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Original Article

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Metabolic effects of amino acid solutions infused for renal protection during therapy with radiolabelled somatostatin analogues

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Abstract

Background. Infusion of amino acids (AAs) can reduce renal uptake of radiolabelled somatostatin analogues resulting in a lower kidney exposure during peptide radiotherapy of patients with neuroendocrine tumours. In this study, we investigated the metabolic effects related to the infusion of large amounts of amino acids in patients undergoing positron emission tomography (PET) studies with [^{86}Y]DOTA^{0}-D-Phe^{1}-Tyr^{3}-octreotide.

Methods. Twenty-four patients, in four consecutive groups of six, received a 4 h infusion of 120 g of mixed AAs and, in addition, either a 4 h infusion of 50 g of L-lysine ($n=6$), a 10 h infusion of 240 g of mixed AAs ($n=6$), a 4 h infusion of 50 g of L-lysine + L-arginine (Lys-Arg; $n=6$) or no infusion (control; $n=6$) in randomly ordered crossover studies. A number of clinical and biochemical parameters in blood and urine were measured over 24 h, including calculation of creatinine clearance, tubular reabsorption of inorganic phosphate (TRP) and fractional urate excretion.

Results. No clinical side effects occurred during the infusions except for nausea and vomiting under mixed AAs. Patients in the latter group showed an increase in serum urea, whereas patients receiving L-lysine showed an increase in serum potassium and chloride. Inorganic phosphate levels dropped at 2.5 h in all groups except controls, and a significant decrease in TRP was observed with mixed AAs but not with L-lysine or Lys-Arg.

Conclusion. Although infusion of AA solutions can improve the effect of therapy by allowing the administration of higher doses of radiolabelled somatostatin analogues, each preparation has specific sides effects that should be taken into account with this type of therapy.

Keywords: amino acids; hyperkalaemia; renal uptake reduction; side effects

Introduction

Neuroendocrine tumours express high affinity somatostatin receptors that enable scintigraphic imaging using radiolabelled somatostatin analogues. Moreover, the opportunity to deliver doses directly to tumour cells showed favourable effects on tumour growth, and produced tumour shrinkage in patients [1–3]. The radiolabelled peptides are filtered by the renal glomerulus and, following interaction with anionic charges on cell membranes, radioligands or their radiometal-chelated (^{111}In, ^{90}Y) metabolic products are trapped in the proximal tubular cells where they become a radiation source for the nearby highly radiosensitive glomeruli [4,5]. This internal irradiation may cause renal thrombotic microangiopathy and represents a serious limitation for the therapeutic use of such agents [6,7]. This limitation justified the interest in different modalities of renal protection during peptide radiotherapy, a therapeutic approach that is becoming applied more often nowadays. It was demonstrated as early as in 1977 that infusion of positively charged amino acids (AAs) such as ornithine, lysine or arginine was able to inhibit tubular reabsorption of low molecular weight proteins [8]. Pre-clinical studies showed that co-infusion of compounds able to produce a defect of renal tubular transport, such as maleate, arginine and lysine, can reduce the renal uptake of radiolabelled peptides in the rat [9]. In patients, intravenous administration of AA solutions showed a reduction of renal uptake of [^{111}In]pentetreotide [10] and [^{99m}Tc]Fab'

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fragments [11]. Recently, in a phase I pharmacokinetics and biodistribution study, we evaluated the effect of various AA infusions on the kidney uptake of a positron emitter-labelled somatostatin analogue, [^{86}Y]DOTA^0-D-Phe^1-Tyr^3-octreotide in patients with a neuroendocrine tumour [12]. A 4 h co-infusion of mixed AAs significantly reduced the renal uptake of this compound by a mean of 21%, allowing the use of higher activities in therapy, with a similar safety margin. A similar benefit was observed with infusion of pure L-lysine, whereas the combination of L-lysine and L-arginine was more effective at reducing renal uptake than mixed AAs. Although this protective approach may be beneficial to the renal function in the long term, little information is available about the acute pharmacological effects of the intravenous administration of large amounts of AAs.

In this study, we have investigated a number of clinical and metabolic effects of different infusion regimens of large amounts of AAs in patients with neuroendocrine tumours.

Subjects and methods

Study design and patients

The data presented here are derived from phase I studies aimed at evaluating the pharmacokinetics and biodistribution of a somatostatin analogue labelled with a positron emitter, yttrium-86 (^{86}Y), namely [^{86}Y]DOTA^0-D-Phe^1-Tyr^3-octreotide (used as a surrogate of the therapeutic agent [^{90}Y]DOTA^0-D-Phe^1-Tyr^3-octreotide; [^{90}Y]SMT487), in patients with neuroendocrine tumours. All patients were eligible for treatment with [^{90}Y]SMT487.

A total of 48 studies were analysed in 24 patients (15 males and nine females; median age 51 years, range 32–65) undergoing comparative evaluations with two different regimens of renal protection. The inclusion criteria included age over 18 years; a plasma creatinine lower than 150 μmol/l or measured creatinine clearance >60 ml/min; no history of congestive heart failure; and no current treatment with potentially nephrotoxic drugs (e.g. aminoglycosides, some antineoplastic chemotherapy agents). Other drugs were allowed when clinically needed, including non-steroidal anti-inflammatory drugs (NSAIDs). Hypertension ($n = 4$), previous renal disease (e.g. infection, stone disease or obstruction) and diabetes mellitus ($n = 5$) were not exclusion criteria unless uncontrolled at the time of enrolment. Patients under total or partial parenteral nutrition were excluded from the studies, but no specific dietary restrictions were applied to the enrolled patients. Patients were recruited in four consecutive groups, each comparing two infusion regimens. Each group of six individuals underwent studies in random order with and without a 4 h infusion of commercially available mixed AAs (G1); with 4 h mixed AA and L-lysine infusion (G2); with 4 and 10 h mixed AA infusion (G3); or with 4 h mixed AA and L-lysine + L-arginine (Lys-Arg) infusion (G4). Randomization within each group was done before the start of the study. The study was approved by the Ethics Committee of the University of Louvain Medical School, and all patients gave written informed consent.

Radiopharmaceuticals

SMT487 (Novartis Pharma AG, Basel, Switzerland) was available as a lyophilized kit containing 80 μg of SMT487, 16 mg of gentisic acid, 40 mg of inositol and 80 mg of ascorbic acid. Yttrium-86 was produced by irradiation of enriched $^{86}SrCO_3$ and radiolabelling was performed by incubation for 20 min at 100°C of the peptide kit with ^{86}Y-Cl_3 in 0.02 M HCl as described previously [12,13].

Amino acid infusions

Commercially available AAs were used: 1.5 l of Proteinsteril Hepa 8% (Fresenius, Bad Homburg, Germany) containing 10.72 g of L-arginine and 6.88 g of L-lysine per litre together with other essential and non-essential AAs (see details in Table 1) was brought to 1.8 l by addition of 300 ml of lactate Ringer solution (Hartmann®, Travenol Laboratories, Lessines, Belgium). The final osmolality was 660 mOsm/l. A supplement of 40 mEq of phosphate (glucose-1-phosphate, Phocytan®, Vanden Bogaerde, Brussels, Belgium) was added in G2, G3 and G4 to the mixed AA solution to limit the drop of serum phosphate observed in G1 (see Results). The volume of injected solution was doubled when mixed AA infusion was prolonged to 10 h (G4). For L-lysine infusion (G2), 1 l of 6.25% L-lysine hydrochloride (Baxter Clintec, Montargis, France) was brought to 1.8 l by addition of 800 ml of lactate Ringer solution. For Lys-Arg infusion (G4), 500 ml of 6.25% L-lysine hydrochloride and 152 ml of 20% L-arginine hydrochloride (BUFA, Uitgeest, The Netherlands) were brought to 1.8 l by addition of 1150 ml of lactate Ringer. Since lactate Ringer contains calcium but no magnesium, $MgSO_4$ (3 g) was added to all infusion bags as the current practice in our institution for standard total parenteral nutrition to avoid hypomagnesaemia. The final osmolality was ~800 mOsm/l.

Infusion schemes are detailed in Table 1. All infusions started 30 min before the injection of [^{86}Y]SMT487. The choice of the mixed AA solution as the reference regimen

Table 1. Schedules for amino acid infusions

	Infusion periods (h)	Infusion rate (gr/h)	Volume (ml)	Total AAs (g)	Total L-lys + L-arg (g)
Mixed AAs 4 h	0–0.5	40	1800	120	26.4
	0.5–1.5	40			
	1.5–4	24			
L-Lysine 4 h	0–0.5	16.7	1800	50	50
	0.5–1.5	16.7			
	1.5–4	9.98			
Mixed AAs 10 h	0–0.5	40	3600	240	52.8
	0.5–1.5	40			
	1.5–4	24			
	4–10	20			
Lys-Arg 4 h	0–0.5	16.7	1800	50	50
	0.5–1.5	16.7			
	1.5–4	9.98			

Concentration of amino acids in the mixed AA solution (g/l in the final solution) were: tryptophan, 0.58; isoleucine, 8.66; threonine, 3.66; phenylalanine, 0.73; valine, 8.4; leucine, 10.90; methionine, 0.92; lysine, 5.73; histidine, 2.33; arginine, 8.93; serine, 1.86; cysteine, 0.43; alanine, 3.86; and proline, 4.78. See 'Study design' for definition of groups.

in the study was made because they were commercially available. Dosages of mixed AAs, L-lysine and Lys-Arg were chosen from previous experience in the literature [8,10]. The initial infusion rate was higher than the final infusion rate in order to reach the highest concentration of amino acids in the tubules at the time of radiotracer injection. No sham infusion was used in controls, but all patients were invited to drink plenty of water during the studies to reduce the retention time of the radiopharmaceutical in the urinary tract, especially in the bladder. Patients were not fasting before or during the studies and none of them were clinically dehydrated.

Data collection

During the study, patients were monitored for clinical side effects, electrocardiographic (ECG) abnormalities, changes in vital signs (blood pressure, heart rate, body temperature, body weight, respiratory rate and diuresis) and blood chemistry. Blood samples were taken just before and 2.5 and 24 h after starting the infusion; in G2–G4, additional samples were obtained at 5, 7 and 16 h post-infusion. Serum creatinine, urea, uric acid, sodium, potassium, bicarbonate, chloride, total calcium and inorganic phosphate were measured by automatic analysis (Hitachi 917 or Hitachi 717; Roche, Vilvoorde; Belgium). In G1, creatinine clearance over 24 h was measured. In G2–G4, fractionated urine collections (0–1, 1–3.5, 3.5–6, 6–24 and 24–48 h) were obtained using an automatic analyser (Axon, Bayer; Belgium) for the measurement of creatinine, urea clearance and total proteinuria, as well as to calculate the tubular reabsorption of phosphate (TRP) and fractional urate excretion. Baseline measured creatinine clearance was not obtained in all patients: when missing, a calculated creatinine clearance using the Cockcroft formula was used for comparison.

Statistical analysis

Intra-individual comparisons were performed using the Student's paired *t*-test and comparisons between groups using the two-sample Student's *t*-test. The statistical significance of the difference in all comparisons was determined on the basis of a two-tailed 5% α-error.

Results

Clinical signs

Infusions were generally well tolerated. No local side effects were observed at the injection site. Some patients complained of nausea and sometimes vomiting after the start of mixed AA infusion (seven out of 24 and two out of six, respectively, when 4 or 10 h infusions were administered). L-Lysine infusion and Lys-Arg infusion were better tolerated with less frequent episodes of nausea and/or vomiting (one out of six in G2 and G4 *vs* four out of six and two out of six, respectively, with mixed AAs). Table 2 details the grade of nausea and vomiting according to the World Health Organization (WHO) toxicity criteria. No other cardiac or renal side effects were noted. No statistically significant changes in vital signs (especially body weight) and no ECG abnormalities, especially in QRS shape and duration and QT interval, were noted (data not shown).

Table 2. Gastrointestinal toxicity according to the WHO

	4 h mixed AAs $n=24$	10 h mixed AAs $n=6$	4 h L-lysine $n=6$	4 h Lys-Arg $n=6$
Nausea				
Grade 0	17	4	6	5
Grade 1	1	0	0	0
Grade 2	4	2	0	1
Grade 3	2	0	0	0
Grade 4	0	0	0	0
Vomiting				
Grade 0	19	4	5	5
Grade 1	2	0	0	0
Grade 2	3	2	1	0
Grade 3	0	0	0	0
Grade 4	0	0	0	0

Toxicity criteria for nausea: grade 0 = none; grade 1 = able to eat reasonable intake; grade 2 = intake significantly decreased but can eat; grade 3 = no significant intake; grade 4 = no intake.
Toxicity criteria for vomiting: grade 0 = none, grade 1 = once in 24 h; grade 2 = 2–5 in 24 h; grade 3 = 6–10 in 24 h; grade 4 = more than 10 in 24 h, or requiring intravenous support.

Biochemistry

Biochemical results observed for each AA regimen, at baseline and after 2.5 and 24 h are shown in Table 3. The effects of AA infusion at 2.5 h were assessed within each group (intra-patient) by normalizing the biochemical data at baseline to unity (Table 4). The most remarkable feature was a significant drop in serum inorganic phosphate in all except the control group at 2.5 h post-infusion. The effect of the AA infusion in reducing serum phosphate was obvious in G1; in this group, this reduction was more marked ($P < 0.03$ *vs* no infusion) as compared with the other groups, with return to baseline values at 24 h post-infusion. Phosphate supplementation of the mixed AA solution in G2, G3 and G4 studies did not totally prevent this effect, although the drop was less marked. Serum phosphate dropped at 2.5 h post-infusion by a mean of 43% (from a mean of 3.3 to 1.9 mg/dl) in patients in the G1 group when they received mixed AAs and by a mean of 21% (from a mean of 3.9 to 3.1 mg/dl) in the 18 patients of G2–G4 under the same regimen when phosphate was added to the infusions ($P < 0.01$). Moreover, the reduction of serum phosphate seemed more prolonged when mixed AAs were administered for 10 h (intra-patient analysis, Figure 1); this was true at 7 h post-infusion ($P < 0.01$) but, in the group who received 10 h infusion, serum phosphate returned to baseline values at 16 h post-infusion. In contrast to phosphate, only a marginal decline in serum calcium was noted. Serum urea increased at 2.5 h post-infusion when mixed AAs were administered during 4 or 10 h. This rise remained statistically significant at 24 h

Table 3. Biochemical results (mean ± SD) at baseline, 2.5 h and 24 h post-infusion in controls and after various regimens of AA infusion

	Phosphate (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	K^+ (mmol/l)	HCO_3^- (mmol/l)	Na^+ (mmol/l)	Uric acid (mg/dl)	Total calcium (mg/dl)	Cl^- (mmol/l)
Controls ($n=6$)									
Baseline	3.1 ± 0.4	37.2 ± 8.9	1.2 ± 0.3	3.8 ± 0.2	23.9 ± 4.0	140.5 ± 3.7	7.5 ± 2.2	8.9 ± 1.2	104.7 ± 7.1
2.5 h	3.2 ± 0.5	39.5 ± 12.5	1.1 ± 0.3	4.2 ± 0.9	22.3 ± 2.3	140.2 ± 2.2	7.3 ± 1.9	8.5 ± 0.7	104.8 ± 4.8
24 h	3.5 ± 0.7	39.5 ± 12.4	1.1 ± 0.3	3.9 ± 0.3	24.8 ± 2.5	140.0 ± 2.9	7.7 ± 1.6	9.0 ± 0.7	102.3 ± 5.4
4 h mixed AAs ($n=24$)									
Baseline	3.8 ± 1.0	33.6 ± 8.1	1.0 ± 0.2	4.2 ± 0.4	28.0 ± 3.3	139.7 ± 2.9	5.7 ± 2.1	9.6 ± 0.7	103.9 ± 3.6
2.5 h	2.8 ± 1.0*	45.8 ± 9.5	0.9 ± 0.3*	4.4 ± 0.6	26.4 ± 3.7	135.0 ± 4.7*	4.8 ± 1.9*	8.9 ± 0.5*	99.3 ± 4.3*
24 h	3.6 ± 0.9	45.4 ± 10.0*	1.0 ± 0.2*	4.0 ± 0.4	26.3 ± 2.7*	137.6 ± 3.3*	5.1 ± 1.8*	9.3 ± 0.5*	101.3 ± 4.5*
4 h L-lysine ($n=6$)									
Baseline	3.6 ± 0.5	33.2 ± 3.8	1.0 ± 0.2	4.1 ± 0.4	27.3 ± 2.5	136 ± 4.4	5.2 ± 1.7	9.7 ± 0.8	99.8 ± 7.1
2.5 h	2.8 ± 0.6*	32.7 ± 2.3	0.9 ± 0.2*	5.1 ± 0.3*	23.8 ± 2.7*	131.2 ± 4.5*	4.4 ± 1.7	9.1 ± 0.7*	104.8 ± 4.5*
24 h	3.6 ± 0.3	34.5 ± 5.6	1.0 ± 0.1	4.1 ± 0.2	21.5 ± 2.2*	134.2 ± 3.3	5.2 ± 2.2	9.7 ± 0.7	101 ± 4.4
10 h mixed AAs ($n=6$)									
Baseline	3.2 ± 0.6	32.0 ± 4.4	1.0 ± 0.1	4.2 ± 0.4	28.7 ± 2.3	141.6 ± 2.2	4.6 ± 1.9	9.8 ± 0.4	104.8 ± 2.6
2.5 h	2.5 ± 0.3*	43.7 ± 5.0*	1.0 ± 0.1*	4.6 ± 0.3	26.6 ± 1.3	136.6 ± 1.8*	4.1 ± 1.8*	9.1 ± 0.4*	101.8 ± 1.8
24 h	3.4 ± 0.3	56.3 ± 17.1*	1.0 ± 0.1	4.0 ± 0.5	27.7 ± 3.1	139.8 ± 1.1	4.2 ± 1.9	9.3 ± 0.5	102.5 ± 2.7
4 h Lys-Arg ($n=6$)									
Baseline	3.3 ± 0.7	33.7 ± 6.1	0.9 ± 0.3	3.8 ± 0.3	26.7 ± 2.7	138.3 ± 2.4	4.7 ± 0.8	9.5 ± 0.8	104.8 ± 2.8
2.5 h	2.3 ± 0.7*	39.8 ± 6.7*	0.8 ± 0.2*	5.1 ± 0.7*	24.8 ± 1.5	135.5 ± 2.7	4.0 ± 0.7	9.0 ± 0.8	109.0 ± 4.0*
24 h	3.5 ± 0.6	38.5 ± 8.3	0.9 ± 0.2	4.2 ± 0.3	26.5 ± 2.3	135.7 ± 2.2*	4.4 ± 0.4	9.7 ± 0.6	103.8 ± 3.5

*$P < 0.05$ *vs* baseline (paired Student's *t*-test).
Values are reported in conventional units (conversion factors for SI units are: 0.323 for phosphate, 0.357 for urea, 88.4 for creatinine, 0.059 for uric acid and 0.249 for total calcium).

Table 4. Intra-patient analysis: biochemical results at 2.5 h post-infusion in patients undergoing comparative studies with various regimens of AA infusion

	G1—no infusion *vs* 4 h mixed AAs ($n=6$)	G2—4 h mixed AAs *vs* L-lysine ($n=6$)	G3—4 h mixed AAs *vs* 10 h mixed AAs ($n=6$)	G4—4 h mixed AAs *vs* 4 h Lys-Arg ($n=6$)
Phosphate	1.04 ± 0.13 *vs* 0.56 ± 0.11*	0.77 ± 0.08 *vs* 0.80 ± 0.07	0.78 ± 0.09 *vs* 0.78 ± 0.09	0.71 ± 0.14 *vs* 0.71 ± 0.11
Urea	1.06 ± 0.14 *vs* 1.30 ± 0.11	1.45 ± 0.16 *vs* 0.97 ± 0.12*	1.42 ± 0.08 *vs* 1.37 ± 0.09	1.36 ± 0.16 *vs* 1.19 ± 0.07*
Creatinine	0.77 ± 0.30 *vs* 0.89 ± 0.43	0.92 ± 0.08 *vs* 0.94 ± 0.06	0.95 ± 0.07 *vs* 0.95 ± 0.04	0.95 ± 0.12 *vs* 0.93 ± 0.06
K^+	1.04 ± 0.22 *vs* 1.12 ± 0.08	0.97 ± 0.22 *vs* 1.24 ± 0.08	0.98 ± 0.17 *vs* 1.12 ± 0.17	1.17 ± 0.13 *vs* 1.34 ± 0.12*
HCO_3^-	0.97 ± 0.22 *vs* 0.98 ± 0.12	0.90 ± 0.10 *vs* 0.88 ± 0.06	0.88 ± 0.11 *vs* 0.93 ± 0.07	1.04 ± 0.06 *vs* 0.94 ± 0.10
Na^+	1.00 ± 0.02 *vs* 0.96 ± 0.07	0.95 ± 0.04 *vs* 0.96 ± 0.01	0.97 ± 0.02 *vs* 0.96 ± 0.02	0.97 ± 0.04 *vs* 0.97 ± 0.01
Uric acid	0.99 ± 0.14 *vs* 0.83 ± 0.09*	0.84 ± 0.10 *vs* 0.84 ± 0.08	0.85 ± 0.07 *vs* 0.87 ± 0.10	0.81 ± 0.07 *vs* 0.86 ± 0.06
Cl^-	1.00 ± 0.07 *vs* 0.95 ± 0.01	0.95 ± 0.05 *vs* 1.04 ± 0.03*	0.97 ± 0.04 *vs* 0.97 ± 0.03	0.94 ± 0.03 *vs* 1.04 ± 0.02*

Results are shown as relative to baseline (mean ± SD).
*$P < 0.05$ paired Student's *t*-test.

post-infusion but was abolished at the follow-up visit at days 7–10. Urea was only modestly increased with Lys-Arg. Conversely, the serum creatinine showed a transient decrease during infusions.

The most relevant observation during L-lysine or Lys-Arg infusion was the increased serum potassium at 2.5 h post-infusion ($P < 0.001$ compared with baseline), with individual values well in excess of 5 mmol/l in 10 out of 12 patients (maximum value 6.2 mmol/l with Lys-Arg). However, hyperkalaemia was not associated with clinical signs or ECG changes. The increment of serum potassium was associated with an increased serum chloride and decreased bicarbonate levels. Similar observations were found when performing comparisons within each group (Table 4).

Urine analysis

No statistically significant reduction of fractional creatinine clearance over 24 h in G1 and up to 48 h in G2–G4 was noted. On the contrary, infusion of AAs seemed to result in a slight increase of the creatinine clearance during the infusion (Table 5). A transient increase of the urea clearance was also noted during infusions of AAs, with a subsequent reduction when the infusions were stopped. Both 4 h mixed AA and L-lysine infusion induced a proteinuria that was more pronounced with L-lysine than with mixed AAs ($P < 0.01$ at 1, 3.5 and 6 h, respectively; Figure 2).

The intra-patient analysis showed a reduction of fractional TRP when mixed AAs were infused. No

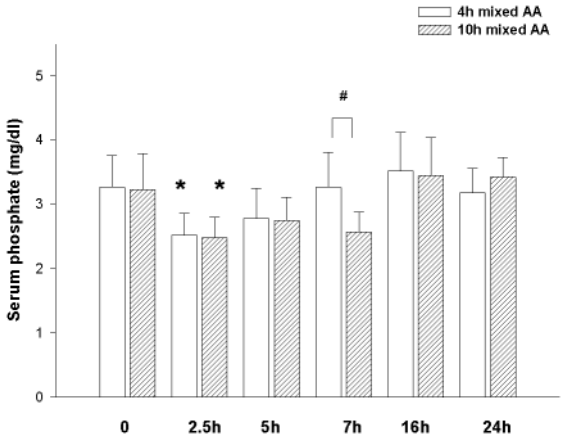

Fig. 1. Serum phosphate (mean ± SD) over time in the same six patients receiving either 4 h (open bars) or 10 h (striped bars) mixed AA infusion. #Statistically significant difference between the two regimens (at 7 h post-infusion, $P < 0.01$). *Statistically significant *vs* baseline ($P < 0.05$).

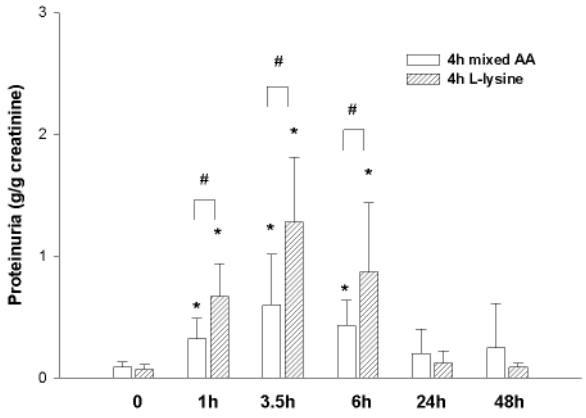

Fig. 2. Mean (± SD) proteinuria (expressed as g/g creatinine to take dilution effects into account) at baseline and in fractionated urine collections obtained at 0–1, 1–3.5, 3.5–6, 6–24 and 24–48 h post-infusion in the same six patients receiving a 4 h infusion of either mixed AAs (open bars) or L-lysine (striped bars). #Statistically significant difference between the two regimens ($P < 0.05$). *Statistically significant *vs* baseline ($P < 0.05$).

change in TRP was noted when L-lysine or Lys-Arg was infused (Figures 3A and B). Reduction of fractional TRP was also noted in patients undergoing the 4 h *vs* 10 h infusion regimens (Figure 3C). However, beyond 3.5 h of infusion (i.e. when the dose rate of AA infusion was reduced), the effect was not sustained in the 10 h group. An increase of the fractional urate excretion was observed with mixed AA, L-lysine (Figure 4) and Lys-Arg infusion (not shown). However, this increase was statistically significant only with L-lysine at 1 h post-infusion.

Discussion

In this study, we analysed the acute clinical and biochemical toxicity induced by infusion of large amounts of AAs given for renal protection. The main findings of the study are: (i) the relatively limited clinical side effects; (ii) the lack of effect on the glomerular filtration rate (GFR); (iii) the increase of serum urea with mixed AAs; (iv) the hyperkalaemia induced by infusions of lysine; and (v) the asymptomatic hypophosphataemia observed under all regimens.

AA infusions were generally well tolerated, although nausea and/or vomiting frequently occurred when a mixed AA solution was employed. Infusion of mixed AAs decreases the lower oesophageal sphincter (LES) pressure and inhibits gastric emptying [14]; this could be responsible in part for the upper gastrointestinal side effects associated with this infusion. This effect could be mediated by L-arginine (via the production of nitric oxide [15] or a direct effect on the central nervous system) or by the aromatic AAs, tryptophan and phenylalanine, through a direct effect on the gastric parietal cells [15]. Little improvement of the symptoms was noted with the use of anti-emetic agents, given on an individual basis for symptomatic control, either those with peripheral action (metoclopramide, alizapride) or the serotonin receptor antagonist, tropisetron. Our more recent experience suggests that domperidone, an anti-emetic agent that increases the LES pressure, might be more effective in this respect. Others also reported gastrointestinal side effects after an infusion rich in arginine with [^{90}Y]DOTATOC therapy [16]. Nausea and vomiting occurred in 50 and 69% of patients depending on the concentrations of arginine and/or lysine in the solution, whereas only 10% of

Table 5. Changes in creatinine clearance (ml/min) (mean ± SD) over time measured from fractionated urine collections showing a trend to increase although differences are not statistically significant in most groups

	Baseline	0–24 h with AAs	0–24 h without AAs			
G1	80.8 ± 39.3	94.7 ± 39.4	87.7 ± 44.8			
	Baseline	0–1 h	1–3.5 h	3.5–6 h	6–24 h	24–48 h
G2 mixed AAs	80.4 ± 22.6	95.7 ± 55.1	109.2 ± 73.2	81.3 ± 25.7	74.8 ± 25.0	86.8 ± 20.7
G2 L-lysine	80.4 ± 22.6	110.8 ± 59.1	101.5 ± 27.6*	83.0 ± 29.3	83.2 ± 30.6	82.8 ± 24.1
G3 4 h mixed AAs	109.4 ± 15.2	122.5 ± 21.3	118.7 ± 29.7	115.0 ± 24.9	99.1 ± 12.9*	107.5 ± 18.2
G3 10 h mixed AAs	109.4 ± 15.2	118.2 ± 6.5	124.5 ± 42.7	118.2 ± 47.0	110.0 ± 21.0	103.6 ± 17.0
G4 mixed AAs	100.6 ± 42.5	114.4 ± 28.9	114.1 ± 33.5	112.7 ± 49.7	111.3 ± 50.7	101.3 ± 24.0
G4 Lys-Arg	100.6 ± 42.5	125.0 ± 51.4	128.6 ± 32.7*	99.7 ± 46.3	102.9 ± 18.9	103.2 ± 54.5

*$P > 0.05$ paired Student *t*-test *vs* baseline.

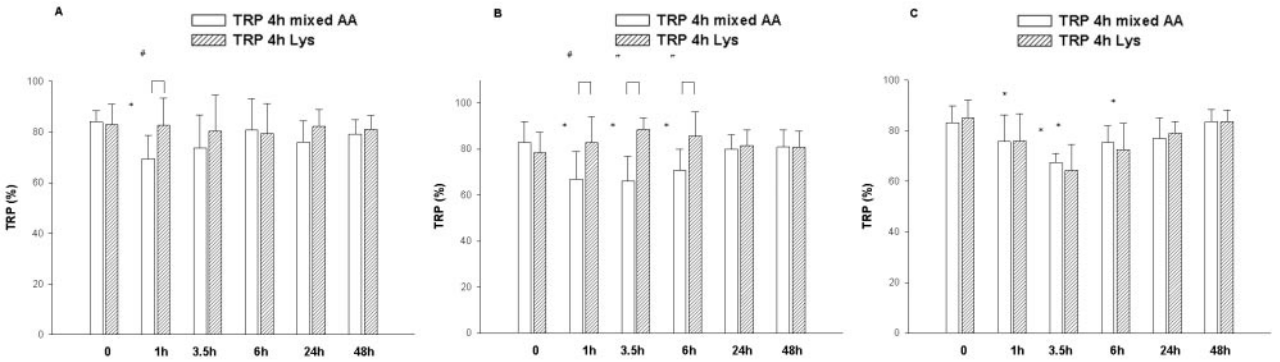

Fig. 3. Changes in tubular reabsorption of phosphate (TRP; mean ± SD) over time. (**A**) A 4 h infusion of either mixed AAs (open bars) or L-lysine (striped bars). (**B**) A 4 h infusion of either mixed AAs (open bars) or Lys-Arg (striped bars). (**C**) An infusion of mixed AAs for either 4 h (open bars) or 10 h (striped bars). *Statistically significant *vs* baseline ($P < 0.05$). #Statistically significant differences between regimens ($P < 0.05$).

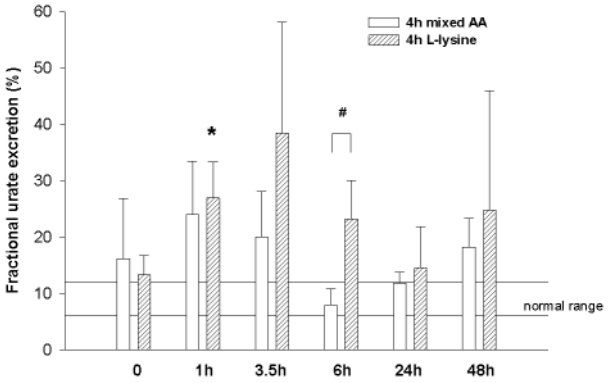

Fig. 4. Fractional urate excretion (mean ± SD) over time in patients receiving a 4 h infusion of either mixed AAs (open bars) or L-lysine (striped bars). *Statistically significant *vs* baseline ($P < 0.05$). #Statistically significant differences between regimens ($P < 0.05$).

patients had symptoms with lysine infusion alone. Similar data were reported in patients treated with [^{111}In]DTPA-octreotide and different AA solutions [17]. The higher incidence of gastrointestinal side effects in the above-mentioned studies compared with our study might be due to the higher doses of radioactivity administered.

No case of acute renal failure was observed and no deleterious effect of the infusions on the GFR, assessed by the creatinine clearance or plasma creatinine, occurred. Variations in body weight were minimal and do not suggest any dehydration during or following infusions. However, a significant increase of serum urea was observed with 4 or 10 h infusion of mixed AAs. This was not reported after pure L-lysine infusion and reverted to baseline values by days 7–10. The commercially available mixed AA solution contains large amounts of L-arginine (10.72 g/l) that is the most involved amino acid in the urea cycle. This metabolic pathway could explain the increase of serum urea due to the overload of the urea cycle after mixed AA infusion. This hypothesis is also supported by the observation of increased serum levels of urea and products of the urea cycle such as ornithine and citrulline after Lys-Arg infusion.

Hyperkalaemia was observed after the administration of 50 g of L-lysine (or 25 g in the Lys-Arg solution). Since lysine is a ketogenic AA [18], the increased kalaemia is probably related to an extracellular shift of potassium secondary to an increased production of ketonic bodies in an acidic environment. However, the acute acidic conditions, illustrated by the bicarbonate and chloride values at 2.5 h post-infusion, could also be related in part to the administration of L-lysine in the hydrochloride form. Our data confirm those of Rolleman *et al.* [17] who reported hyperkalaemia in some patients infused with lysine or Lys-Arg solution. It must be noted that there was a moderate elevation of serum potassium in our series that was not associated with substantial ECG changes. Nevertheless, the potential cardiac risk must be recognized [19] and should be monitored by ECG, particularly in patients with cardiac diseases including arrhythmia. It is possible that the use of the D-stereoisomer, which does not interfere with physiological AA metabolism, would allow this potentially severe side effect to be reduced.

Asymptomatic, transient hypophosphataemia was also noted during amino acid infusion. Reduction of serum phosphate might be related to an increased anabolism, described as refeeding hypophosphataemia [20]; to an intracellular shift related to acidosis; or to a decreased reabsorption of phosphorus by a non-specific tubular cell blockade. The reduction of TRP after mixed AAs supports the latter hypothesis.

Fractionated urine analysis showed that infusions caused a transient proteinuria that was not evaluated further (i.e. qualitatively) since AA infusion is known to induce non-selective proteinuria [9] in this therapeutic setting, reflecting the inhibition of proximal tubule endocytosis. L-Lysine appeared to have a more pronounced effect on proteinuria (Figure 2). This probably reflects the specific efficiency of cationic AAs to inhibit tubular reabsorption of several substances including proteins and peptides. It must, however, be kept in mind that the amount of L-lysine used alone (50 g) was

larger than the total amount of cationic AAs in the mixed AA regimen (26.4 g). Since urate is an anionic molecule, the increased urate excretion during AA infusion could not be explained by competition with the cationic AAs on proximal tubular cells. Extracellular fluid volume expansion has a major influence in renal handling of urate and could explain the increased urate excretion during the infusion. The findings of an increased phosphate and urate excretion, transient hyponatraemia, proteinuria and hypophosphataemia are suggestive of a generalized tubular dysfunction when large amounts of AAs are administered.

In conclusion, we showed that the intravenous administration of large amounts of AAs given for renal protection floods the renal tubule system and impairs its function in terms of both protein reabsorption and anion balance. These biochemical effects were totally reversible within 24 h (or 7 days in the case of urea with mixed AAs). It is worth noting that patients with chronic renal disorders and patients at high risk of potential renal dysfunction (e.g. patients under chemotherapy, total parenteral nutrition or with congestive heart failure) were not enrolled in the study. In general, and more especially in such patients, the biochemical changes observed following infusion of large amounts of AAs should be considered in the planning of radiopeptide therapy, taking into account not only the benefit derived by the increase of the administered activities to patients but also the acute risk that could be associated with them.

Conflict of interest statement. None declared.

References

1. Krenning EP, Kooij PPM, Bakker WH *et al*. Radiotherapy with a radiolabelled somatostatin analogue [^{111}In-DTPA-D-Phe^{1}]-octreotide. *Ann NY Acad Sci* 1994; 733: 496–506
2. de Jong M, Valkema R, Jamar F *et al*. Somatostatin receptor-targeted radionuclide therapy of tumors: preclinical and clinical findings. *Semin Nucl Med* 2002; 32: 133–40
3. McCarthy KE, Woltering EA, Espanan GD, Cronin M, Maloney TJ, Anthony LB. *In situ* radiotherapy with ^{111}In-pentetreotide: initial observations and future directions. *Cancer J Sci Am* 1998; 4: 94–102
4. Behr TM, Goldenberg DM, Becker W. Reducing the renal uptake of radiolabeled antibody fragments and peptides for diagnosis and therapy: present status, future prospects and limitations. *Eur J Nucl Med* 1998; 25: 201–212
5. Akizawa H, Arano Y, Uezono T, *et al*. Renal metabolism of ^{111}In-DTPA-D-Phe^{1}-octreotide *in vivo*. *Bioconjug Chem* 1998; 9: 662–670
6. Cybulla M, Weiner SM, Otte A. End-stage renal disease after treatment with ^{90}Y-DOTATOC. *Eur J Nucl Med* 2001; 28: 1552–1554
7. Moll S, Nickeleit V, Mueller-Brand J *et al*. A new cause of renal thrombotic microangiopathy: yttrium 90-DOTATOC internal radiotherapy. *Am J Kidney Dis* 2001; 37: 847–851
8. Mogensen CE, Sølling K. Studies on renal tubular protein reabsorption: partial and near complete inhibition by certain amino acids. *Scand J Clin Lab Invest* 1977; 33: 477–486
9. de Jong M, Rolleman EJ, Bernard BF *et al*. Inhibition of renal uptake of indium-111-DTPA-octreotide *in vivo*. *J Nucl Med* 1996; 37: 1388–1392
10. Hammond PJ, Wade AF, Gwilliam ME *et al*. Amino acid infusion blocks renal tubular uptake of an indium-labeled somatostatin analogue. *Br J Cancer* 1993; 67: 1437–1439
11. Behr TM, Becker WS, Sharkey RM *et al*. Reduction of renal uptake of monoclonal antibody fragments by amino acid infusion. *J Nucl Med* 1996; 37: 829–833
12. Jamar F, Barone R, Mathieu I *et al*. ^{86}Y-DOTA^{0}-D-Phe^{1}-Tyr^{3}-octreotide (SMT487)—a phase 1 clinical study: pharmacokinetics, biodistribution and renal protective effect of different regimens of amino acid co-infusion. *Eur J Nucl Med* 2003; 30: 510–518
13. Rösch F, Herzog H, Stolz B *et al*. Uptake kinetics of the somatostatin receptor ligand [^{86}Y]DOTA-D-Phe^{1}-Tyr^{3}-octreotide ([^{86}Y]SMT487) using positron emission tomography in non-human primates and calculation of radiation doses of the ^{90}Y-labeled analogue. *Eur J Nucl Med* 1999; 26: 358–366
14. Gielkens HAJ, Lamers CBHW, Masclee AAM. Effect of amino acids on lower esophageal sphincter characteristics and gastroesophageal reflux in humans. *Dig Dis Sci* 1998; 43: 840–846
15. Straathof JWA, Adamse M, Onkenhout W, Lamers CBHW, Masclee AAM. Effect of L-arginine on lower oesophageal sphincter motility in man. *Eur J Gastroenterol Hepatol* 2000; 12: 419–424,
16. Bodei L, Cremonesi M, Zoboli S *et al*. Receptor-mediated radionuclide therapy with 90Y-DOTATOC in association with amino acid infusion: a phase I study. *Eur J Nucl Med* 2003; 30: 207–216
17. Rolleman EJ, Valkema R, de Jong M, Kooij PPM, Krenning EP. Safe and effective inhibition of renal uptake of radiolabelled octreotide by a combination of lysine and arginine. *Eur J Nucl Med* 2003; 30: 9–15
18. Lehninger AL, Nelson DL, Cox MM. Amino acid oxidation and the production of urea. In: *Principles of Biochemistry*, 2nd edn. Worth Publishers, New York; 1993: 506–537
19. Weiner ID, Wingo CS. Hyperkalemia: a potential silent killer. *J Am Soc Nephrol* 1998; 9: 1535–1543
20. Weisinger JR, Bellorín-Font E. Magnesium and phosphorus. *Lancet* 1998; 352: 391–396

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