Effect of feed supplementation with phytohaemagglutinin in combination with α-ketoglutarate on growth and nitrogen elimination pathways in rats with acute renal failure induced by nephrectomy

Rafał Filip[1,2], Adrian P. Harrison[3], Stefan G. Pierzynowski[1]

[1]Department of Cell and Organism Biology, Lund University, Lund, Sweden
[2]Institute of Agricultural Medicine, Lublin, Poland
[3]Department of Animal and Veterinary Basic Sciences, Faculty of Life Science, Copenhagen University, Frederiksberg C, Denmark

Submitted: 19 March 2008
Accepted: 21 May 2008

Arch Med Sci 2008; 4, 2: 122–128

Corresponding author:
Dr. Rafał Filip
Department of Endoscopy
Institute of Agricultural Medicine,
Jaczewskiego 2,
20-950 Lublin, Poland
Phone: +48 503 088 433
Fax: +48 81 718 44 70
E-mail: r.s.filip@wp.pl

Abstract

Introduction: Phytohaemagglutinin (PHA) and alpha-ketoglutaric acid (AKG) in growing rats stimulate a change in the proportion of N excretion via urine and faeces, in favour of faecal excretion. The aim of the study was to investigate the effect of oral supplementation of PHA and AKG on pathways of nitrogen excretion and serum levels of urea in uraemic conditions induced by nephrectomy.
Material and methods: Experiment 1 – 12 rats were assigned to one of two groups, control and PHA. Experiment 2 – PHA was administered to 36 male rats which were assigned to 4 groups: 1) uraemic control, 2) uraemic + AKG, 3) Sham-operated, 4) Sham-operated + AKG. AKG was administered via drinking water, while PHA was administered *via* a stomach tube.
Results: Lower daily weight gain ($P<0.05$), increase in small intestine and total GI tract weight ($P<0.05$) as well as significant reduction in N excretion in urine in the PHA group were observed ($P<0.05$). Significantly higher daily weight loss in the uraemic rats, compared to that of the sham-operated rats, was observed ($P<0.05$). A significant increase in N excretion in faeces was observed in the AKG group, compared to control within the sham-operated rats ($P<0.05$) and when compared to the uraemic rats ($P<0.05$). In both sham-operated and uraemic rats, AKG treatment led to a significant reduction in the urea levels ($P<0.05$).
Conclusions: The change in the proportion of N excretion via urine and faeces caused by PHA due to increasing the rate of protein production in the intestinal wall, apparently favouring faecal excretion, can be enhanced by the oral administration of AKG.

Key words: alpha-ketoglutaric acid, phytohaemagglutinin, nitrogen elimination, intestine, Rat, ARF.

Introduction

Despite improvements in critical care and dialysis technology, both acute (ARF) and chronic kidney failure (CRF) remains associated with a significant decrease in the quality of life as well as with high mortality rates. Although there is a positive association between early initiation of dialysis (at lower baseline blood urea nitrogen levels) and lower mortality rates in both ARF and CRF [1], there are potential safety concerns. These safety concerns regarding earlier initiation of dialysis include: increased risk of infection from an indwelling dialysis catheter, hypotension

associated with therapy and its consequences (including the potential for delayed renal recovery), and leukocyte activation from contact with dialysis membranes [2, 3]. Whether these risks outweigh the potential benefits of earlier initiation of dialysis will require individual consideration; therefore, in some cases delaying the pre-dialysis period is the treatment of choice.

Alpha-ketoglutaric acid (AKG), a glutamine derivative, is considered to be one of the crucial molecules in trans-membrane amino acid transportation, protein metabolism, and both gene and cellular redox regulation [4]. Both animal and human studies have shown that 95% of luminal glutamate and 70% of glutamine, compared to only 40% of AKG, is metabolized (first pass) to CO_2 by the intestinal mucosa [5-8]. Deamination of amino acids releasing amino nitrogen for growth results in the production of ammonia, which is generally protonated to ammonium at physiological pH. Ammonia is toxic for the body; therefore it is maintained at a very low level in the blood. In post-weaning pigs, extracellular or intra-mitochondrially generated ammonia may by converted to urea by enterocytes [9]. On the other hand, ammonia itself may be an essential part of the environment necessary for the growth of enterocytes [10]. In support of this, AKG has been shown to exert a beneficial effect on nitrogen metabolism through facilitating ammonium conversion to amino acids and protein, and by reducing levels of ammonium in the body [11]. Moreover, AKG has the potential to restore acid base homeostasis, spare nitrogen, decrease bone turnover and improve renal function in the catabolic acidotic patient, which justifies its application in patients with chronic renal failure (CRF) [12, 13].

The interaction of red kidney bean (*Phaseolus vulgaris*) lectin, phytohaemagglutinin (PHA), with the GI tract is well documented [14, 15]; indeed, it has been shown that PHA accelerates the turnover of GI tract cells. Such rapid cell proliferation might be expected to result in both increased gut growth and functional maturation and, as a further consequence, an increase in the use of dietary amino acids for protein synthesis by enterocytes [15, 16]. Animal studies have shown that over a 3-day period oral administration of PHA to young suckling rats results in gut maturation in terms of epithelial development, decreased lactase but increased maltase and sucrase activities, as well as a reduction in macromolecular absorptive capacity, sometimes referred to as gut closure [17].

Our previous studies have shown that combined administration of PHA and AKG in growing rats stimulates small bowel growth by increasing crypt depth and the weight of the small intestine, resulting in: 1) a higher rate of protein production in the intestinal wall, and 2) an apparent change in the proportion of N excretion via urine and faeces, in favour of faecal excretion [18].

However, the potential for such treatment in uraemic conditions remains untested. Hypothetically, an increased cellular turnover in enterocytes upon addition of PHA, resulting in an increase in ammonia production, in the presence of AKG, will tend to promote its incorporation into amino acids and reduce the level of urea production.

The primary aim of the present study was therefore to investigate the effect of oral supplementation of PHA in combination with AKG on pathways of nitrogen excretion in uraemic conditions induced by nephrectomy. The secondary aim was to study the effect of such treatment on the serum levels of urea.

Material and methods

Rats came from the Sprague-Dawley outbred rat laboratory (M&B A/S, Denmark), and were caged individually in metabolic cages (60% humidity with 12 hours of light per 24 hours) at the Department of Animal Physiology, Lund, Sweden. Rats were given *ad libitum* access to food: a mixture of wheat, barley, extracted soya bean meal, fat blend, mineral, vitamin and amino acid, with a dietary composition (%) Dig. Crude Protein 19.67, Dig. Crude Oil 3.76, calcium 0.65, total phosphorus 0.51 of which 0.24 was available, lysine 1.26 and methionine 0.30, and a Dig. Energy of 13.75 MJ/kg. The vitamin content *per* kg of diet comprised: vitamin A 14 960 IU, vitamin D_3 1574 IU, vitamin E 110.4 mg, thiamin 17.6 mg, riboflavin 13.2 mg, pyridoxine 17.9 mg, vitamin B_{12} 35 µg, vitamin K 19.9 mg, folic acid 3.1 mg, nicotinic acid 34.1 mg, pantothenic acid 34.1 mg, choline 1557.0 mg, inositol 1811.4 mg, biotin 369.2 µg (Table I). The study was approved by the Ethical Review Committee for Animal Experiments at Lund University, and conducted according to European Community regulations concerning the protection of experimental animals.

Groups were equalised with regard to body weight. Treatments and experimental conditions were conducted according to the recommendations of the Federation of the European Laboratory Animal Science Association (FELASA).

Experiment 1

After a 1-week adaptation period, 7-week old male rats with initial body weight of 251.1±1.4 g were assigned to 2 experimental groups and kept in metabolic cages for 10 days: 1) control, 2) PHA, with 6 animals in the control group, and 6 in the treatment group. A stomach tube was applied in all animals once each day, in the morning, while PHA was administered via a stomach tube only in the PHA group. The stock solution of crude PHA in 0.9% NaCl was (20% w/v) in water: 50 mg PHA/ml, 20 ml/kg body weight.

Table I. Composition of diet fed to rats for 10 days (experiments 1 and 2). Main ingredients: wheat, barley, extracted soya bean meal, fat blend, minerals, vitamin premix[a], amino acids. Composition of experimental drinks (experiment 2)

Calculated dietary composition (experiments 1 and 2)	
Dig. Crude Protein	19.67%
Dig. Crude Oil	3.76%
Dig. energy [MJ/kg]	13.75%
Calcium	0.65%
Total phosphorus	0.51%
Available phosphorus	0.24%
Lysine	1.26%
Methionine	0.30%

Composition of experimental drinks (experiment 2)		
	Control	AKG
AKG	–	146 g
Glucose	300 g	300 g
Sucrose	150 g	150 g
NaOH	36 g	36 g
KOH	7.5 g	7.5 g
$Ca(OH)_2$	4.6 g	4.6 g
$Mg(OH)_2$	1.8 g	1.8 g
HCl	75 ml	–

[a]*Providing the following per kilogram of diet (g/kg diet): vitamin A 14960 IU, vitamin D_3 1574 IU, vitamin E 110.4 mg, thiamin 17.6 mg, riboflavin 13.2 mg, pyridoxine 17.9 mg, vitamin B_{12} 35 µg, vitamin K 19.9 mg, folic acid 3.1 mg, nicotinic acid 34.1 mg, pantothenic acid 34.1 mg, choline 1557.0 mg, inositol 1811.4 mg, biotin 369.2 µg*

Collection of GI tract

At the end of the trial, rats were euthanized with an overdose of pentobarbital; stomach, proximal and distal small intestine, caecum and colon were collected and weighed.

Experiment 2

36 male rats at age of 17 weeks and initial body weight of 317.55±18.34 g were randomly assigned to 4 experimental groups: 1) U-control (uraemic control), 2) U-AKG (uraemic with AKG treatment), 3) S-Control (sham-operated control), 4) S-AKG (sham-operated with AKG treatment), with 9 animals in each group. PHA was administered via a stomach tube to all animals (groups 1-4). A stock solution of crude PHA in 0.9% NaCl was administered (20% w/v) in water: 50 mg PHA/ml, 20 ml/kg body weight. AKG was administered via the drinking water (Table I). Control rats were administered HCl in the drinking water to compensate the acidic effect of the AKG preparation (Table I).

Surgical procedure

Uraemic rats (U-Control and U-AKG) underwent 5/6 nephrectomy from a dorsal approach by the performance of right total nephrectomy and ligation of 2/3 to 3/4 of the arterial supply to the left kidney. At the beginning, part of the left kidney was removed, and 1 week later the right kidney was completely removed. Rats were premedicated with 10-15 mg/kg b. wt. of xylazine and anaesthetized with ketamine 80 mg/kg b. wt. All rats had blood catheters in both jugular veins.

After a 7-day recovery period, rats were placed in metabolic cages for 10 days.

Urine and faeces sampling

Collection of urine into a cup was performed every day, with 10 ml of a 50% H_2SO_4 solution being used to stop microbial activity, while faeces, which were also collected in a cup, were kept dry. The total volume of urine was measured, and a homogenised sample of 10 ml was collected for urea analysis. The weight of the faeces was also measured on a daily basis, followed by collection into 60 ml of a 50% H_2SO_4 solution. At the end of the experiment, the total collection of faeces was analysed for nitrogen content.

Analysis of total nitrogen

Nitrogen content was measured on a Leco Nitrogen and Protein Determinator FP-428 (Leco Corporation. St. Joseph. MI., USA). The instrument was set up in low range mode. Prior to analysis, the faecal samples were homogenised (Sorvall Omni-Mixer). The faecal samples, 150-200 mg, were analysed in tin foil cups. The urine samples (100-200 µl) were analysed in tin capsules measuring 15 × 6 mm (length/diameter). To obtain urine samples for nitrogen determination, aliquots of 1% of the daily urine volume were pooled. A glycine standard solution containing 1% nitrogen was used as a reference. All nitrogen determinations were made on duplicate samples.

Calculations and statistics

Total feed intake was calculated from the amount of food used per day, although an approximation was made to compensate for any feed which entered the cup used for collecting faeces. This correction factor was found to be constant for all treatments and amounted to 10.77% of the total feed provided. The final feed intake was therefore adjusted using this factor. The nitrogen level of the feed was calculated from the level of protein in the feed – 19.76%. Data, which were normally distributed and of equal variance, were analysed for statistical significance between means using Student's t-test. In all statistical analyses $P<0.05$ was considered significant.

Results

Performance

Experiment 1 – the average starting weight of the control group was 248.19 g, and the body weight of the treated group was not significantly different from that of the controls. The results showed significantly lower daily weight gain as well as final body weight (data not shown) for the PHA group, compared to the controls (P<0.05) (Table II). Both water and feed intake were similar in the PHA and control group (Table II).

Experiment 2 – the average starting weights of the control groups (uraemic and sham-operated) were 304.08 and 337.50, respectively; and the body weight of the treated groups was not significantly different from that of the controls. The results showed a significantly higher daily weight loss in the uraemic rats (P<0.05), compared to that of the sham-operated rats (Table III). Surprisingly, within the uraemic groups, a significantly higher daily weight loss was observed in the AKG group compared to the control group (P<0.05) (Table III). The final body weight was also found to be lower in uraemic groups (P<0.05, data not shown).

Urinary and faecal N

In experiment 1, a significant reduction in urine production was found between the controls and the PHA treated rats, although no difference in the total level of excreted faeces was found between the two groups (Table IV).

In experiment 2, the results showed an increase in the level of urine production in uraemic rats, although with statistical significance visible only in the control group (P<0.05). There was no difference between treatments with respect to the faeces excreted after 10 days of trial (P<0.05) (Table III).

In experiment 1, a significant reduction in N excretion in urine was observed in the PHA group, compared to the control group (41% lower, P<0.05). There was no difference between treatments with respect to the N excretion in faeces (Table IV).

Calculation of the relative level of N in faeces to the total N intake (faecal N/intake N%) showed that in the PHA treated animals the percentage of N excreted in faeces increased, compared to the control group, in favour of N excretion *via* the faeces. This was also visible after calculating the percentage of N excreted in faeces in relation to the N excreted in both faeces and urine [faecal N/(urine N + faecal N)%] (Table IV).

In experiment 2, no significant difference in N excretion in urine was observed in the uraemic rats, compared to the sham-operated rats. However, in the uraemic rats administered AKG, a numerical reduction (trend) of N excretion was observed, compared to control. A significant increase in N excretion in faeces was observed in the AKG group, compared to control within the sham-operated rats (P<0.05) and when compared to the uraemic rats (P<0.05).

Calculation of the relative level of N in faeces to the total N intake (faecal N/intake N%) showed that in both sham-operated and uraemic groups, in AKG treated animals the percentage of N excreted in faeces increased, compared to the control group, in favour of N excretion via the faeces. In sham-

Table II. Mean daily growth performance, feed and water intake, excretion of urine and faeces in young rats (experiment 1)

Measurements	Control	PHA
Daily gain [g]	5.1±0.5	3.6±0.5*
Feed intake [g]#	19.9±2.7	21.3±0.9
Water intake [ml]	48.2±8.5	36.4±6.1
Urine [ml]	29.8±7.6	18.5±4.2*
Faeces [g]	9.7±1.4	11.7±2.3

*Values are means ± SEM. Significance of changes against control (Student's t-test): *P<0.05*
#Correction was made to compensate for any feed which entered the cup used for collecting faeces (≈10.77% of total feed provided)

Table III. Mean daily growth performance, feed and water intake, excretion of urine and faeces in rats in sham-operated and uraemic rats administered PHA (stomach tube, 50 mg/kg b. wt.) (experiment 2)

Measurements	Sham-operated rats		Uraemic rats	
	control	AKG	control	AKG
Daily gain [g]*	–1.1±0.4[a]	–1.1±1.5[a]	–1.7±0.83[b]	–3.7±0.87[c]
Feed intake [g]*,#	16.18±3.2[a]	13.4±2.4[a]	17.9±1.6[a]	13.8±2.2[a]
Water intake [ml]*	59.4±8.4[a]	57.1±10.6[a]	61.5±5.8[a]	64.8±15.6[b]
Urine [ml]*	43.1±6.0[a]	39.2±3.5[a]	55.4±2.6[b]	45.0±3.7[a]
Faeces [g]*	20.6±2.1[a]	21.3±0.9[a]	20.2±1.0[a]	17.3±1.4[a]

Values are means ± SEM
**Within a row, values with different letters are different (P<0.05, Student's t-test)*
#Correction was made to compensate for any feed which entered the cup used for collecting faeces (≈10.77% of total feed provided)

Table IV. Mean daily intake and excretion of nitrogen (N) in young rats (experiment 1)

Measurements	Control	PHA
Intake of N in feed [g/day][&]	0.63±0.20	0.57±0.21
N in urine [g/day]	0.43±0.21	0.25±0.06*
N in faeces [g/day]	0.13±0.07	0.14±0.07
N for growth [g/day][$]	0.13±0.03	0.09±0.02*
N balance [g]	0.07	0.17
Urine N/ intake N [%]	68.25	43.85
Faecal N/ intake N%	20.63	24.56
Faecal N/ (urine N + faecal N) [%]	23.21	35.9

*Values are means ± SEM. Significance of changes against control (Student's t-test): *P<0.05*
[&]Calculated level of consumed N = [total feed consumption × protein level in feed (0.1967) × true protein dig. (0.868)]/6.25
[$]Calculated level of N lost (negative body weight gain) = [total weight, g × level of protein in flesh (0.16)]/6.25

Table V. Mean daily intake and excretion of nitrogen (N) in sham-operated and uraemic rats administered PHA (stomach tube, 50 mg/kg b. wt.) (experiment 2)

Measurements	Sham-operated rats		Uraemic rats	
	control	AKG	control	AKG
Intake of N in feed [g/day]*,[&]	1.63±0.15[a]	1.73±0.12[a]	1.68±0.07[a]	1.29±0.07[b]
N in urine [g/day]*	1.31±0.12[a]	1.31±0.09[a]	1.29±0.06[a]	1.17±0.12[a]
N in faeces [g/day]*	0.40±0.04[a]	0.45±0.01[b]	0.40±0.01[a]	0.35±0.03[a]
N in urine [g/d]*- N lost [g][$]	1.28	1.28	1.25	1.07
N balance [g]	–0.08	–0.03	–0.01	–0.23
Urine N/ intake N [%]	78.5	73.9	74.4	82.9
Faecal N/ intake N%	24.5	26.0	23.8	27.1
Faecal N/ (urine N + faecal N) [%]	23.8	26.0	24.2	24.6
Urea in serum [mmol/l][day 0]	5.20±0.43[a]	5.50±0.65[a]	7.68±0.66[b]	10.38±1.41[c]
Urea in serum [mmol/l][day 10]	4.43±0.95[a]	3.80±0.15[b]	7.09±0.40[c]	7.44±1.16[c]
Urea[day 0] – urea[day 10]/urea[day 10] [%]	–14.7	–30.9	–7.68	–28.3

Values are means ± SEM
**Within a row, values with different letters are different (P<0.05, Student's t-test)*
[&]Calculated level of consumed N = [total feed consumption × protein level in feed (0.1967) × true protein dig. (0.868)]/6.25
[$]Calculated level of N lost (negative body weight gain) = [total weight, g × level of protein in flesh (0.16)]/6.25

operated rats this was also visible after calculating the percentage of N excreted in faeces in relation to the N excreted in both faeces and urine [faecal N/(urine N + faecal N)%] (Table V).

Serum urea levels

Experiment 1 – in general, in both sham-operated and uraemic rats, AKG treatment led to a significant reduction in the urea levels (30.9 and 28% reduction in sham-operated and uraemic rats, respectively – P<0.05) (Table V).

Gastro-intestinal tract weights

Experiment 1 – in general, the changes influenced by the treatment were related to the small intestine and large intestine. However, in the PHA group, a statistically significant weight increase was observed with regard to the small intestine and total GI tract, compared to that of the control group (P<0.05) (Table VI).

Table VI. GI tract weights in young rats (experiment 1)

GI tract parts	Control	PHA
Stomach [g]	1.3±0.04	1.4±0.14
Small Intestine [g]	6.4±0.28	8.7±0.46*
Large Intestine [g]	1.1±0.09	1.3±0.07
Caecum [g]	1.1±0.07	1.3±0.11
Total GIT [g]	10.1±0.37	12.8±0.57*

*Values are means ± SEM. Significance of changes against control: *P<0.05*

Discussion

Effect of PHA and AKG on rat performance

Plant lectins bind avidly to the mucosal surface and induce dose- and time-dependent as well as fully reversible hyperplastic and hypertrophic growth in the small intestine [15]. The underlying mechanism for the PHA effect is most likely the same as for some peptide growth factors and hormones – it binds to cell surface receptors of the brush border membrane, and acts as an extraneous growth factor in the gut. By interacting with brush border epithelial receptors, PHA induces extensive proliferation and changes in the metabolism of epithelial cells by activation of second messenger pathways [15].

AKG, after oral administration, is rapidly removed from the systemic circulation with a half-life of under 5 min [19]. Only 40% of AKG is taken up and metabolized to CO_2 in the intestinal mucosa in the first pass [7], the remainder generally following common metabolic pathways via glutamate to other amino acids (e.g. proline). In the intestine, glutamate is channelled towards proline (and ornithine) synthesis (*via* glutamic-γ-semialdehyde) because of AKG derived saturation of the oxidative needs of the enterocytes [20].

In experiment 1, PHA treatment negatively influenced rat performance, which at least in part supports previous findings showing that the ingestion of PHA (from red kidney beans) leads to a reduction in body weight, and at high doses may damage the gut mucosa. However, lectin at low doses (0.01-0.2 g/kg BW) does not exert a strong anti-nutritional effect [15] and may be one of the most powerful growth factors in the rat alimentary tract [21, 22], e.g. even a 10-day oral PHA treatment induces dose dependent growth in the small intestine and pancreas. Our results pertaining to the weight of the GI tract generally substantiate the above- mentioned findings [4, 17, 21, 22] in that the weight of the small intestine was significantly increased with PHA treatment. This was also confirmed by calculation of the ratio of total body weight/GI tract weight, which was higher in PHA treated animals (data not shown).

In experiment 2, not surprisingly, the induction of renal failure negatively influenced body performance [23], which may not be explained only by the lower feed intake since uraemic and sham-operated control rats ate the same amount of the fed mixture. In might be instead attributed to the influence of AKG. One might think that it is quite surprising, because even if AKG caused the reduction of the rat appetite, on the other hand it is also a strong anabolic factor which was itself capable of compensating the reduction in the intake of feed (and loss of weight). However, when taking into consideration that the AKG was administered in combination with PHA, this phenomenon could have a rational explanation, e.g. that AKG was used as a scavenger of ammonium and after its conversion to amino acids and proteins (with the simultaneous effect of reducing the levels of ammonium) was utilised in the intestinal wall due to the increased cell production caused by PHA.

Effect of PHA and AKG on urinary and faecal N balance

The results of experiment 1 showed that PHA treatment alters the proportion of N excreted in urine compared to that of faeces, so that faecal N excretion appears to be favoured, which is consistent with our previous studies [18]. The reduction of the urine excretion in PHA treated rats was not followed by the reduction of N excretion via urine, and therefore may be attributed to the reduced water intake. It is well known that under physiological conditions, water intake has no influence on total urea (and nitrogen) excretion *via* the kidneys [24].

In experiment 2, in which PHA was administered to rats from all study groups, the proportion of N excreted in urine compared to that of faeces was also altered in favour of N excretion; however, this effect was more visible in sham-operated rats, compared to the uraemic rats. The most probable explanation is the lowest intake of N in feed observed in uraemic rats administered AKG. The highest weight loss followed by N loss was also observed in that particular group; however, it must be pointed out that a negative nitrogen balance was present in all study animals.

The observed trend indicating the altered proportion of N excreted in urine compared to that in the faeces was such that faecal N excretion appears to be favoured in rats administered PHA in combination with AKG s, and supports previous studies in which AKG treatment had a beneficial impact on the level of glutamate metabolised by enterocytes in the proximal small intestine [8, 20]. However, it must be pointed out that according to our previous experiments, the addition of AKG alone to the diet did not exert any influence on the amount of N excreted in the urine or faeces. This leads to the conclusion that AKG tends to enhance the effect of PHA, or exerts its effect only in the presence of PHA. The underlying mechanism is complex and not yet clearly elucidated.

In 1951, it was shown in a study of potassium accumulation by rabbit kidney slices that oxygen consumption per hour per mg wet weight of tissue increased by 209% compared to control levels upon addition of AKG [25], which provides clear evidence of a significant increase in cellular metabolism after exposure to AKG. These results, combined with the results of Bardocz [15] and those of Linderoth et al. [17] showing PHA stimulated growth of the small intestine to be

achieved through an elevated rate of cellular production, come closer to explaining the beneficial role of combined AKG and PHA administration.

Dietary management offers a new dimension of the therapeutic approach to the complex of interrelated symptoms of uraemia. The essence of this approach is to restrict protein intake while allowing adequate quantities of total calories and essential amino acids (and keto-analogues) to maintain nitrogen equilibrium. Generally, it is recommended that daily ingestion of protein should be limited to 0.5-0.6 g/kg of body weight plus an amount equal to urinary protein losses. Although the therapeutic results of different diets are very promising, the level of renal function at which these protein-restricted diets lose their effectiveness in maintaining patients relatively symptom-free varies from individual to individual. The clinical response to the protein-restricted diet appears to be related to age, physical condition, presence of other diseases, and the attitudes and emotional strength of the patient. Therefore, additional modification of the dietary treatment by adding factors capable of enhancing the excretion of N by faeces, rather than urine, could be an interesting option.

In conclusion, the results show that the change in the proportion of N excretion via urine and faeces caused by PHA due to increasing the rate of protein production in the intestinal wall, apparently favouring faecal excretion, can be enhanced by the oral administration of AKG. This effect is also present in the conditions of uraemia, which reveals some new perspectives for subjects suffering from renal failure.

References

1. Uchino S, Kellum JA, Bellomo R, et al. Acute renal failure in critically ill patients: A multinational, multicenter study. JAMA 2005; 294: 813-8.
2. Conger J. Vascular alterations in ARF: establishment and maintenance. In: Acute Renal Failure. 1st ed. Molitoris BA, Finn W. New York, Saunders WB 2001; 13-29.
3. Teehan GS, Liangos O, Lau J, Levey AS, Pereira BJ, Jaber BL. Dialysis membrane and modality in acute renal failure: understanding discordant meta-analyses. Semin Dial 2003; 16: 356-60.
4. Linderoth A, Prykhod'ko O, Pierzynowski SG, Weström BR. Enterally but not parenteralny administered Phaseolus vulgaris lectin induces growth and precocious maturation of the gut in suckling rats. Biol Neonate 2006; 89: 60-8.
5. Wernerman J, Hammarqvist F. Modulation of endogenous glutathione availability. Curr Opin Clin Nutr Metab Care 1999; 2: 487-92.
6. Le Bricon T, Coudray-Lucas C, Lioretn N, et al. Ornithine alpha-ketoglutarate metabolism after enteral administration in burn patients: bolus compared with continuous infusion. Am J Clin Nutr 1997; 65: 512-8.
7. Junghans P, Derno M, Pierzynowski SG, Hennig U, Souffrant WB. Utilization of a-ketoglutarate (AKG) in young growing pigs after intra-venous and intra-duodenal administration. The EAAP – Symposium (European Association for Animal Production) on Energy and Protein Metabolism and Nutrition, Rostock-Warnemuende, Germany, September 2003; 521-3.
8. Lambert BD, Filip R, Stoll B, et al. First-pass metabolism limits the intestinal absorption of enteral alpha-ketoglutarate in young pigs. J Nutr 2006; 136: 2779-84.
9. Wu G. Urea synthesis in enterocytes of developing pigs. Biochem J 1995; 312: 717-23.
10. Rhoads JM, Argenzio RA, Chen W, et al. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. Am J Physiol 1997; 272: G943-53.
11. Wirén M, Permert J, Larsson J. Alpha-ketoglutarate – supplemented enteral nutrition: effects on postoperative nitrogen balance and muscle catabolism. Nutrition 2002; 18: 725-8.
12. Welborn JR, Shpun S, Dantzler WH, Wright SH. Effect of alpha-ketoglutarate on organic anion transport in single rabbit renal proximal tubules. Am J Physiol 1998; 274: F165-74.
13. Filip RS, Pierzynowski SG, Lindegard B, Wernerman J, Haratym-Maj A, Podgurniak M. Alpha-ketoglutarate decreases serum levels of C-terminal cross-linking telopeptide of type I collagen (CTX) and preserves bone mass in postmenopausal women with osteopenia: six-month study. Int J Vit Nutr Res 2007; 77: 89-97.
14. Pusztai A, Ewen SW, Grant G, et al. Relationship between survival and binding of plant lectins during small intestinal passage and their effectiveness as growth factors. Digestion 1990; 46 Suppl. 2: 308-16.
15. Bardocz S. Effect of phytohaemagglutinin on intestinal cell proliferation. Role of polyamines. Arch Latinoam Nutr 1996; 44 (4 Suppl. 1): S16-20.
16. Linderoth A, Prykhod'ko O, Ahrén B, Fåk F, Pierzynowski SG, Weström BR. Binding and the effect of the red kidney bean lectin, phytohaemagglutinin, in the gastrointestinal tract of suckling rats. Br J Nutr 2006; 95: 105-15.
17. Linderoth A, Prykhod'ko O, Pierzynowski SG, Weström BR. Enterally but not parenteralny administered Phaseolus vulgaris lectin induces growth and precocius maturation of the gut in suckling rats. Biol Neonate 2006; 89: 60-8.
18. Filip R, Harrison A, Bieńko M, Radzki RP, Pierzynowski SG. Dietary supplementation with phytohemagglutinin in combination with alpha-ketoglutarate limits the excretion of protein via the urinary tract in rats. 2007 in press.
19. Dabek M, Kruszewska D, Filip R, et al. Alpha-ketoglutarate (AKG) absorption from pig intestine and plasma pharmacokinetics. J Anim Physiol Anim Nutr 2005; 89: 419-26.
20. Kristensen NB, Jungvid H, Fernandez JA, Pierzynowski SG. Absorption and metabolism of alpha-ketoglutarate in growing pigs. J Anim Physiol Anim Nutr (Berl) 2002; 86: 239-45.
21. Rådberg K, Biernat M, Linderoth A, Zabielski R, Pierzynowski SG, Weström BR. Enteral exposure to crude red kidney bean lectin induces maturation of the gut in suckling pigs. J Anim Sci 2001; 79: 2669-78.
22. Banwell JG, Howard R, Kabir I, Arian TE, Diamond RH, Abramowsky C. Small intestinal growth caused by feeding red kidney bean phytohemagglutinin lectin to rats. Gastroenterology 1993; 104: 1669-77.
23. Neu AM, Fivush BA, Warady BA, et al. Longitudinal analysis of intermediate outcomes in adolescent hemodialysis patients. Pediatr Nephrol 2003; 18: 1172-6.
24. Samarasinghe S, Vokes T. Diabetes insipidus. Expert Rev Anticancer Ther 2006; 6 (Suppl. 9): S63-74.
25. Mudge GH. Studies on potassium accumulation by rabbit kidney slices: Effect of metabolic activity. Am J Physiol 1951; 165: 113-27.