Short time L-glutamine supplementation of malnourished rats[1]

Suplementação de ratos desnutridos com L-glutamina por tempo curto

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ABSTRACT

Objective

Considering that in malnourished patients supplemented with L-glutamine the negativity of the nitrogen balance tends to decrease, the present study aimed to determine whether malnourished Wistar rats supplemented with L-glutamine would show lower urinary nitrogen excretion, a greater deposition of nitrogen content in different tissues, and/or an alteration in the plasma amino acid levels.

Methods

The rats were divided into groups: 1) protein-energy malnutrition, 2) protein malnutrition and 3) normally nourished group. The urinary and tissue nitrogen contents were determined by the Kjeldahl method and plasma amino acids by liquid chromatography.

Results

Weight, urinary and tissue nitrogen accumulation were significantly reduced in the group with protein-energy malnutrition, but did not improve with L-glutamine supplementation supplied for a short time. The plasma amino acid concentrations showed no special pattern with L-glutamine supplementation.

Conclusion

It was concluded that it was not possible to detect any positive effect of L-glutamine supplementation on the tissue and urinary nitrogen metabolism in malnourished rats.

Indexing terms: amino acids, dietary supplements, glutamine, malnutrition, nitrogen, rats.

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RESUMO

Objetivo

Considerando que em pacientes desnutridos suplementados com L-glutamina, a negatividade do balanço de nitrogênio tende a diminuir, o presente estudo visou determinar se a suplementação com L-glutamina em ratos Wistar desnutridos poderia diminuir a excreção de nitrogênio urinário, melhorar a deposição de nitrogênio em diferentes tecidos e/ou alterar as concentrações plasmáticas de aminoácidos.

Métodos

Os animais foram divididos em grupos: 1) desnutrido protéico-energético, 2) desnutrido protéico e 3) nutrido. O nitrogênio urinário e tecidual foi determinado pelo método de Kjeldahl e as concentrações plasmáticas de aminoácido por cromatografia líquida.

Resultados

Pesos, nitrogênio urinário e teciduais foram significativamente reduzidos no grupo com desnutrição protéico-energética, não tendo sido encontrada melhora no grupo com suplementação aguda de glutamina. Não foram observadas mudanças nas concentrações plasmáticas de aminoácidos com a suplementação de glutamina.

Conclusão

Não foi possível detectar um efeito positivo da suplementação de glutamina sobre o metabolismo de nitrogênio urinário e tecidual de ratos desnutridos.

Termos de indexação*: aminoácidos, suplementos dietéticos, glutamina, desnutrição, nitrogênio, ratos.*

INTRODUCTION

Protein-energy malnutrition (PCM) is defined by the World Health Organization[1] as a pathological condition resulting from a deficient supply of energy and protein. PCM occurs in about 35% of the hospitalized patients[2]. In Brazil, the prevalence of hospital malnutrition is 40%-60%[2,3]. In addition to offering standard diet therapy[4], several attempts have been made to supplement patients with specific amino acids[5,6] such as alanine, lysine and glutamine. In parallel, both the amounts of energy and protein offered are implicated in a greater or lesser urinary excretion of nitrogen, that may cause a negative nitrogen balance[7,8], aggravating PCM.

L-glutamine (Gln), NH_2-C(O)-CH_2-CH_2-CH-NH_2-COOH, is the most abundant amino acid in the plasma of mammals and, since it is synthesized in practically all tissues, is considered to be non-essential[9,10]. Two nitrogen groups, amino and amide, are characteristic of Gln, causing it to be important for nitrogen transport between the various tissues and for ammonia transport from the periphery to the visceral organs[11,12]. In addition to participating in protein and peptide structures, Gln is also a precursor of gluconeogenesis, of renal ammoniogenesis and neurotransmitters such as α-aminobutyric acid and glutamate, also providing nitrogen for the synthesis of purines, pyrimidines and nucleotides[13,14]. Considering the multiple functions of Gln in the organism, its use has been recommended as an adjuvant in both enteral and parenteral diet therapy for malnourished patients[13,15,16] and for low birth weight infants[17]. However, no effect of Gln on protein turnover was observed in healthy people or dogs[15,18].

The aim of the present study was to determine the effect of supplementation with Gln (0.42g/kg/day) offered through a gastric tube (gavage), for 3 days, on urinary excretion and tissue incorporation of protein nitrogen, and on the plasma amino acid levels of malnourished rats. The possibilities of enriching dietary sources with Gln and the clinical usefulness of such supplementation for the treatment of PCM were considered.

METHODS

Thirty non-obese Wistar rats at 7-8 months of age, weighing 500-550 g, obtained from the

Central Animal House of the School of Medicine of *Ribeirão Preto*, *São Paulo* University (USP), fed with chow diet (19.9g protein/% and 3.71kcal/g Nuvilab® Cr-1, Nuvital Nutrientes, Ltda., Colombo, Brazil), were used for the present protocol. They continued on the chow diet for another 7 days, considered as the control period, in individual cages. These animals were then evenly divided, at random, into 5 groups, receiving different supplies of energy and protein throughout the experiment.

Group (N): with 6 animals, considered as the nourished control rats, ingested 74kcal diet/day and 4g of protein /day (14%) for 25 days.

Group (PCM): 6 animals, called the protein-energy malnutrition group, received 50.0% less total food for 25 days, that is, 37kcal/day and 2 g protein/day (6.5%).

Group (PCMRGln): 6 animals received 50% less total food for 21 days, similar to the PCM group. Then on days 22, 23 and 24 they received 65%-75% of the total food plus Gln supplementation (see below).

Group (PCMGln): 6 animals received 50% less total food for 21 days, similar to the PCM group. Then on days 22, 23 and 24 they mantained with the same amount of food plus Gln supplementation (see below).

Group (PMGln): 6 animals received 100.0% the caloric diet (similar to Group N), but only half of the amount of nitrogen, that is 2g of protein/day (6.5%), for 21 days. Then on days 22, 23 and 24 they also received Gln supplementation (see below).

Gln supplementation was supplied through a gastric tube (gavage) at the rate of 0.42g/kg/day, equivalent to 0.12g of nitrogen (Dipeptiven®, Fresenius Kabi Brazil, Ltda *Campinas- São Paulo*) for 3 days. On the 25th day the animals from the five groups were sacrificed for sample collection (urine, tissue nitrogen and plasma amino acid concentrations).

Twenty-four hour urine samples were also collected on the 1st, 7th, 14th, 21st and 25th days for total nitrogen determination (Kjeldahl micromethod)[19]. To kill the animals, on the last day of the experiment, they were anesthetized with 99% tribromoethanol (Aldrich Chemical Company, Inc. Milwaukee, USA) at the rate of 1mL/kg body weight. Peripheral blood (1mL into a heparinized tube) for the analysis of the plasma amino acids by liquid chromatography and tissue was then collected. Laparotomy for removal of the viscera was performed by way of an abdominal median longitudinal incision. The following tissues were collected: 1) muscle: 2cm fragment of abdominal muscle located on the left side of the paramedial wall; 2) intestinal tissue: the duodenojejunal ligament was localized and the jejunum was measured up to a point located approximately 10cm distally, and removed[20], 3) the left kidney; 4) the whole liver; 5) the heart. The right and left heart ventricles were separated by longitudinal section using the aorta as reference[20]. After removal, the wet tissues were immediately weighed and stored at -70ºC. Total nitrogen was determined in all tissue samples[19] and amino acid concentrations were determined in the plasma[21]. The statistical test occurred on to the results obtained for the first and last days of supplementation. The data were analyzed using the non-parametric Friedman test and multiple comparisons by the Dunn test, with the level of significance set at $p<0.05$.

RESULTS

Experimentally induced malnutrition resulted in 35% weight loss compared to the initial weight in the PCM groups, with no improvement of weight loss after Gln supplementation. However, the PM group (protein malnutrition) maintained its weight throughout the experiment (Table 1).

The data concerning urinary nitrogen excretion over a 24h period are presented in Table 2. In contrast to the weight evolution, the PM group presented an intense fall in urinary nitrogen, especially during the first week of malnutrition. Over the course of the experiment there was a

recovery of the levels of urinary nitrogen excretion. No change in urinary nitrogen was observed in the groups supplemented with Gln, showing that the use of Gln supplementation did not reduce the nitrogen loss of the malnourished animals. In the first days, the PM group has shown a decrease in urinary nitrogen excretion, but did not after receiving glutamine supplementation.

Table 3 shows the nitrogen concentrations per tissue (wet weight). The use of Gln did not lead to a difference in tissue nitrogen between the various groups studied, suggesting that Gln had no effect on tissue nitrogen incorporation in this experimental model. In the PCM group all the tissue showed a decrease in tissue nitrogen, but with no statistically significant difference.

The plasma amino acid concentrations presented in Table 4 showed no consistent pattern. As an example, the alanine levels were significantly

Table 1. Weight of the experimental groups (g).

Group (n)	Basal	7th day	14th day	21st day	25th day
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
N (6)	575 ± 25	467 ± 40	461 ± 41	461 ± 32	471 ± 27[c]
PCM (6)	524 ± 20	470 ± 10	408 ± 16	355 ± 21[a]	320 ± 30
PCMRGln (6)	520 ± 45	463 ± 42	406 ± 43	328 ± 18[b]	327 ± 24[c]
PCMGln (6)	571 ± 33	518 ± 35	470 ± 38	416 ± 40[d]	364 ± 44[d]
PMGln (6)	552 ± 39	530 ± 39	528 ± 40	521 ± 40[ab]	507 ± 36[c]

Data are reported as means±SD; PCM= protein-energy malnutrition; PCMRGln= protein-energy malnutrition re-fed and supplemented with Gln; PCMGln= protein-energy malnutrition supplemented with Gln (no re-feeding); PMGln= protein malnutrition with Gln supplementation; The same letters in the same column or on the same line indicate a significant difference (p<0.05).

Table 2. Urinary nitrogen excreted by the experimental groups (mg/24h).

Group (n)	Basal	7th day	14th day	21st day	25th day
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
N (6)	389 ± 61	378 ± 58	376 ± 43	378 ± 44	380 ± 61
PCM (6)	361 ± 87	233 ± 42	286 ± 81	280 ± 57	283 ± 50
PCMRGln (6)	403 ± 43	311 ± 158	300 ± 121	315 ± 126	360 ± 123
PCMGln (6)	514 ± 103	236 ± 80	270 ± 103	332 ± 68	442 ± 197
PMGln (6)	505 ± 68	130 ± 40	226 ± 54	231 ± 37	398 ± 178

Data are reported as means ± SD; PCM= protein-energy malnutrition; PCMRGln= protein-energy malnutrition re-fed and supplemented with Gln; PCMGln= protein-energy malnutrition supplemented with Gln (no re-feeding); PMGln= protein malnutrition with Gln supplementation.

Table 3. Tissue nitrogen for the various groups at the end of the experiment (x 10^{-3}g N/g tissue, wet weight).

Tissures	N (6)	PCM (6)	PCMRGln (6)	PCMGln (6)	PMGln (6)
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
AM	11 ± 7[a]	3 ± 1	2 ± 0	1 ± 0[ab]	5 ± 0[b]
Fasting	3 ± 1	1 ± 0	1 ± 0[c]	1 ± 0	3 ± 0[c]
Left kidney	7 ± 5[d]	2 ± 0	1 ± 0[d]	2 ± 1	5 ± 1
Total liver	7 ± 4[e]	2 ± 0	2 ± 1[f]	1 ± 0[eg]	8 ± 2[fg]
Right V	3 ± 0[h]	2 ± 0	1 ± 1[j]	1 ± 0[hj]	4 ± 0[lj]
Left V	5 ± 2[l]	2 ± 0	2 ± 0	1 ± 0[lm]	4 ± 1[m]

Data are reported as means±SD; AM= abdominal rectus muscle; V= ventricle; N= nourished; PCM= protein-energy malnutrition; PCMRGln= protein-energy malnutrition re-fed and supplemented with Gln; PCMGln= protein-energy malnutrition supplemented with Gln (no re-feeding); PMGln= protein malnutrition with Gln supplementation; The same letters in the same line indicate a significant difference (p<0.05).

Table 4. Plasma amino acid concentrations for the experimental groups after death (μmol/L).

Amino acids	N (6)	PCM (6)	PCMRGln (6)	PCMGln (6)	PMGln (6)
	M ± SD	M ± SD	M ± SD	M ± SD	M ± SD
Glycine	41 ± 29	60 ± 24	75 ± 42	51 ± 18	72 ± 10
Serine	41 ± 16[a]	55 ± 9	79 ± 26[a]	48 ± 5	58 ± 21
Arginine	57 ± 24[b]	99 ± 19[bc]	77 ± 20[bd]	17 ± 7[d]	29 ± 8[cd]
Tyrosine	35 ± 19[e]	99 ± 27	98 ± 91[ef]	37 ± 16[f]	33 ± 3[f]
Glutamic acid	23 ± 2	33 ± 7	67 ± 7	40 ± 1	27 ± 4
Alanine	136 ± 89[g]	419 ± 61	668 ± 69[g]	141 ± 9	161 ± 7
Aspartic acid	6 ± 1	5 ± 0[h]	5 ± 2	8 ± 0[h]	5 ± 1[h]
Leucine	43 ± 23	95 ± 60	117 ± 76[l]	35 ± 15	29 ± 5[i]
Methionine	30 ± 20	48 ± 14	56 ± 28[j]	14 ± 3[j]	23 ± 3
Valine	88 ± 22[l]	185 ± 71[lm]	134 ± 23	79 ± 14[m]	61 ± 21[m]
Threonine	63 ± 19[o]	94 ± 17	96 ± 48[o]	55 ± 19[o]	98 ± 28

Data are reported as mean±SD; N= nourished; PCM= protein-energy malnutrition; PCMRGln= protein-energy malnutrition re-fed and supplemented with Gln; PCMGln= protein-energy malnutrition supplemented with Gln (no re-feeding); PMGln= protein malnutrition with Gln supplementation; The same letters in the same line indicate a significant difference ($p<0.05$).

higher in the PCMRGln group (animals that received 65% total food and glutamine supplementation after protein caloric restriction), but not in the PCMGln or PMGln groups.

DISCUSSION

Animal mortality during this experiment was 40%, regardless of the experimental group. This mortality rate was similar to that reported in other studies using a similar experimental model[22].

The weight loss observed in the groups with protein-energy malnutrition was probably related to the deficient energy supply. In the group with only protein malnutrition, the weight loss was smaller, suggesting that the maintenance of an energy supply is important to control weight loss. Thus it is suggested that the reduction in energy supply was the major factor responsible for the reduction in body weight and that the supply of Gln did not change this loss.

Urinary nitrogen excretion was significantly reduced in the group with protein malnutrition, starting on the 7th day of the experiment. In parallel, the lower nitrogen loss in the groups with protein-energy malnutrition may suggest that adaptation to the deficiency was more effective among these animals. In addition, supplementation with Gln did not change the rhythm of urinary nitrogen excretion. However, considering that Gln supplementation corresponded to 1/3 of the ingested nitrogen and that nitrogen was not lost in the urine, one can speculate that this nitrogen supply was retained as nonspecific nitrogen, as observed in malnourished animals that received a greater dietary protein supply in addition to Gln supplementation (group PCMRGln). Similarly, Felgines et al.[23], in a rat study on the influence of age in prolonged diet restriction, observed the occurrence of an adaptive mechanism in diet restriction in terms of nitrogen balance and weight loss in 19% rats fasted for 3 days. Hill et al.[24] in a rat study on re-feeding, as also reported by Kirsch et al.[25], also showed that Gln supplementation for 3 days did not affect urinary nitrogen losses.

The overall reduction in tissue nitrogen observed here in the malnourished animals suggests that, with restriction of the protein and energy sources, this nitrogen is rapidly consumed, suggesting animal adaptation to the new catabolic condition. However, tissue nitrogen was not reduced in any of the tissues studied in the presence of protein malnutrition. These results indicate that the energy source may be related to tissue protein degradation. The present data

showed that re-feeding and/or Gln supplementation after malnutrition did not change the concentrations of tissue nitrogen in any of the groups studied. The nitrogen concentrations in the kidney and liver of young nourished rats[26] were similar to those for the N group studied here.

The plasma concentrations of essential and non-essential amino acids showed some differences, without a consistent pattern. As an example, alanine increased after Gln supplementation in the protein-energy deficient group, but did not increase in the protein deficient group. One may consider that this protocol used the alanine-glutamine dipeptide as a Gln carrier. Since alanine increased in one group one would expect the same in other groups, but this did not occur. Protein malnutrition alone is probably responsible for these amino acid alterations, but this remains to be studied in future investigations. These findings may also be due to the increased nitrogen requirements of the different tissues, but may also reflect greater peripheral tissue degradation with a resulting increase in circulating amino acids, both in protein and in protein-energy malnutrition, regardless of Gln supplementation or a greater dietary supply (nonspecific nitrogen). In general, the present results agree with those reported by Anderson et al.[27] and Peng et al.[28], showing that the lower the protein supply the higher the plasma concentration of essential amino acids.

The present study on the effect of Gln supplementation showed no differences in the evolution of weight loss, urinary nitrogen excretion, tissue nitrogen or plasma amino acid concentrations, during the 3 days of Gln supplementation. These results agree with previous studies that showed no relationship between the Gln supply and different aspects of protein metabolism, such as those conducted by Hiramatsu et al.[18] on young eutrophic subjects, by Marchini et al.[15] on eutrophic dogs and by Tavares & Takahashi[29] on rats submitted to gamma radiation. Thus, no beneficial effect of Gln in terms of nitrogen metabolism was observed, even in malnourished animals, suggesting that Gln, specifically, has no influence on the recovery of malnourished animals. Perhaps Gln functioned as a nonspecific nitrogen source and was used as a source of protein nitrogen.

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