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Maillard's reaction in parenteral solutions supplemented with arginine

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SUMMARY. Arginine, as a basic amino acid, can alter the pH of a parenteral solution and consequently to interfere in the stabilility of other amino acids. The objective of this study was to analyze the chemical stability of amino acids in solutions for parenteral nutrition with arginine supplementation. Amino acids concentrations were determined using an amino acid analyzer in intervals of 12, 36, 72 hours, 7 and 28 days. Storage temperatures were -20°C, 5°C and 25°C. A decrease was observed ($p<0.05$) in the concentrations of threonine, methionine, isoleucine, leucine, proline and lysine on the 28th day of study, in the solutions kept at 25°C. The side chains of threonine, methionine, isoleucine, leucine, proline and lysine have hydrophobic groups, which renders them less soluble in water when compared to amino acids that have polar side chains. The degradation of lysine was significant in the sample of parenteral nutrition supplemented with arginine, possibly due to the fact that the solution's pH was between 7.5 and 7.6, therefore facilitating the Maillard reaction between lysine and glucose.
Key words: Parenteral nutrition, amino acids stability, arginine.

RESUMO. Reação de Maillard em soluções parenteral suplementadas com arginina. A arginina sendo um aminoácido básico, pode alterar o pH da solução de nutrição parenteral e consequentemente pode influenciar na estabilidade de outros aminoácidos. O objetivo deste estudo foi verificar a estabilidade química dos aminoácidos em soluções de nutrição parenteral com suplementação de arginina. As concentrações dos aminoácidos foram determinadas no analizador de aminoácidos em intervalos de 12, 36, 72 horas, 7 e 28 dias. As temperaturas de estocagem foram –20°C, 5 °C e 25°C. Foi observado um decréscimo ($p<0.05$) nas concentrações de treonina, metionina, isoleucina, leucina, prolina e lisina no 28 dia de estudo, nas soluções estocadas a 25°C. As cadeias laterais de treonina, metionina, isoleucina, leucina, prolina e lisina tem grupos hidrofóbicos o que torna estes aminoácidos menos solúveis em água quando comparados aos aminoácidos que tem cadeias laterais polares, diminuindo o Ph e a concentração dos aminoácidos na solução, estando mais disponíveis para ragir com a cetona do aldeído da glicose. A degradação de lisina foi significativa nas amostras de nutrição parenteral suplementadas com arginina, possivelmente devido ao pH da solução estar entre 7.5 e 7.6, facilitando a reação de Maillard entre a lisina e a glicose.
Palavras chave: Nutrição parenteral, aminoácidos, estabilidade, arginina.

INTRODUCTION

Arginine is commonly known as a metabolic precursor for creatine, polyamines, nitric oxide and proteins. It also has an important role in the urea cycle. More specifically, arginine shows immune and anabolic effects (1), among which are the stimulation of the hypophyseal growth hormone, the stimulation of anti-tumor actions and the increase of killer and T-helper cells activities (2,3). The supplementation of arginine in parenteral nutrition solutions has been suggested for patients with cancer, because it seens to decrease the frequency of septic complications and the mortality rates in postoperative period (4,5).

The appropriate period and temperature of storage of parenteral nutrition solutions, specially when supplemented with additional amounts of arginine, are controversial. Being a basic amino acid, arginine may alter the solution's pH and thus influence the chemical stability of other amino acids present in parenteral solutions. The unstability of solutions supplemented with arginine could bring direct consequences in the establishment of the validity periods of such solutions.

This study was accomplished with the objective of evaluating the chemical stability of the amino acids threonine, glycine, alanine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, proline and arginine itself in parenteral nutrition solutions suplemented with extra arginine. Storage temperature, pH, presence and absence of light, color of solutions and growth of microorganisms were studied. Influence of other components used in parenteral nutrition formulations on amino acid concentrations were also monitored as control of factors that could interfere in the quantitative analysis of the amino acids.

MATERIAL AND METHODS

Preparation of solutions

Forty eight flasks were divided into six groups of solutions designated by the letters A through F (Table 1). All solutions

were prepared in a horizontal laminar air-flow hood using aseptic technique (6) as recommended by the Brazilian National Coordinating Committee on Large Parenteral Volumes (7).The concentration of amino acids in solutions A and B were compared, but in the flasks A there were 25 g/ litre more arginine than in flasks B. Solutions C, D, E and F were used as controls of factors that could interfere in the quantitative analysis of the amino acids, such as purity and quality of the solution components. The parenteral nutrition solutions did not contain lipids which could interfere in the amino acids analysis.

TABLE 1
Composition of samples

Composition	Solutions					
	A	B	C	D	E	F
Glucose (g/liter)[a]	250.0	250.0	-	-	-	250.0
Amino acids (g/liter)[b]	95.8	95.8	95.8	95.8	-	-
Arginine (g/liter)[c]	35.6	10.6	-	35.6	35.6	-
Sodium chloride (mEq/liter)[d]	68.0	68.0	-	-	-	68.0
Potassium choride (mEq/liter)[d]	13.4	13.4	-	-	-	13.4
Magnesium sulfate (mEq/liter)[d]	20.8	20.8	-	-	-	20.8
Potassium phosfate (mEq/liter)[d]	20.0	20.0	-	-	-	20.0
Calcium gluconate (mEq/liter)[d]	3.0	3.0	-	-	-	3.0
Zinc (2,5 mg/ml)[e]	5.0	5.0	-	-	-	5.0
Copper (0,8 mg/ml)[e]	1.6	1.6	-	-	-	1.6
Manganese (0,4 mg/ml)[e]	0.8	0.8	-	-	-	0.8
Chromium (10,0 mg/ml)[e]	20.0	20.0	-	-	-	10.0

[a] Glucose 50%, 500ml (Fresenius Laboratories, Brazil).

[b] Aminosteryl 10%, without electrolytes, 500ml (Fresenius Laboratories, Brazil), Table 2

[c] Arginine supplemented, 2.5% (Ajinomoto Coorporation of Brazil).

[d] Electrolytes (B. Braum Laboratories, Brazil).

[e] Minerals (Abbott Laboratories, Brazil).

Twelve amino acids were monitored during the study. Table 2 lists the composition of the amino acids solution (Aminosteryl 10% ®) utilized in the study. The influence of temperature and light on amino acid concentrations in the solutions, with and without arginine, were studied by submitting the samples to -20°C, 5°C and 25°C and those which were kept at 25°C under constant fluorescent light or darkness. The pH of the solutions were determined in paralell with amino acid analysis. In this way the conditions of an hospital infirmary were simulated (25°C). Aliquots from all flasks were removed in intervals of 12, 36 and 72 hours, 7 and 28 days and their amino acid concentrations were determined.The color of each solution was observed as an indicative of qualitative degradation of components.

TABLE 2
Composition of amino acids of solution*

Amino acid	Amount (g/liter)
L-Threonine	4.2
Glycine	15.9
L-Alanine	15.0
L-Valine	5.9
L-Methionine	4.1
L-Isoleucine	4.6
L-Leucine	7.0
L-Fenylalanine	4.8
L-Lysine	5.9
L-Histidine	2.8
L-Arginine	10.6
L-Proline	15.0

*Aminosteryl 10% (95.8g/liter), without eletrolytes, Fresenius Laboratories, Brazil.

Microbiological assays

Analysis of microbiological growth were performed at intervals of both zero-time and 28 days. Aliquots were inoculated in thioglycolate and Sabourand culture medium.

Amino acid analysis

Amino acid analysis were performed in a Hitachi L-8500 amino acid analyzer using high resolution ion exchange column and ninhydrin post-column detection. The variation coeficient of amino acid analysis, determined at the beginning and at the end of each analysis was 1.0%.

Statistical analysis

Data were analyzed with Student's *t* test and statistical significance was determined as $p<0.05$.

RESULTS

Significant variations were not found in the amino acids concentrations of solutions with and without supplementation of arginine (samples A and B respectively) storaged at -20°C and 5°C; therefore, the results obtained at -20°C were omitted from Tables 3 and 4. For the purpose of comparison between solutions, 5°C was considered as the ideal storage temperature, since the samples stored at that temperature presented lower amino acid degradation (Tables 3 and 4).

TABLE 3
Average percentage of remaining amino acids in parenteral nutrition solutions, supplemented with arginine (Sample A)

Amino acids	28 days 5°C	25°C Sheltered from light	25°C In presence of light
Threonine*	99.4 ± 0.01	89.0 ± 0.01	87.0 ± 0.01
Glycine	99.5 ± 0.07	98.1 ± 0.10	97.9 ± 0.03
Alanine	99.2 ± 0.01	99.2 ± 0.02	99.6 ± 0.03
Valine	99.8 ± 0.04	99.9 ± 0.01	99.2 ± 0.02
Methionine*	98.6 ± 0.02	88.0 ± 0.08	86.0 ± 0.01
Isoleucine*	99.3 ± 0.01	89.2 ± 0.04	86.0 ± 0.03
Leucine*	98.4 ± 0.03	90.1 ± 0.04	88.0 ± 0.04
Fenilalanine	99.5 ± 0.01	99.3 ± 0.01	99.0 ± 0.01
Lysine*	97.0 ± 0.02	90.0 ± 0.01	87.0 ± 0.01
Histidine	97.4 ± 0.01	96.3 ± 0.03	96.0 ± 0.04
Arginine	99.0 ± 0.05	99.0 ± 0.06	90.0 ± 0.09
Proline*	99.0 ± 0.06	89.5 ± 0.01	87.0 ± 0.07

*Average percentage degraded based on 100% present at zero-time control ±SD, $p<0.05$ (5°C x 25°C, sheltered from light ; 5 °C x 25°C, in presence of light; 25°C, sheltered from light x 25°C, in presence of light).

TABLE 4
Average percentage of remaining amino acids in parenteral nutrition solutions, without arginine supplemented (Sample B)

Amino acids	28 days 5°C	25°C Sheltered from light	25°C In presence of light
Threonine*	96.0 ± 0.02	90.8 ± 0.08	89.8 ± 0.09
Glycine	99.4 ± 0.10	99.1 ± 0.89	98.1 ± 0.09
Alanine	99.2 ± 0.08	99.2 ± 0.53	99.6 ± 0.19
Valine	98.0 ± 0.08	98.6 ± 0.63	97.3 ± 0.05
Methionine*	98.0 ± 0.06	90.7 ± 0.57	88.0 ± 0.09
Isoleucine*	96.8 ± 0.05	90.3 ± 0.50	87.8 ± 0.01
Leucine*	98.1 ± 0.04	97.0 ± 0.64	93.1 ± 0.01
Fenilalanine	99.0 ± 0.01	99.4 ± 0.01	99.3 ± 0.01
Lysine*	99.2 ± 0.07	95.4 ± 0.09	94.1 ± 0.09
Histidine	98.0 ± 0.09	98.2 ± 0.42	98.0 ± 0.05
Arginine	99.1 ± 0.10	99.1 ± 0.70	90.9 ± 0.04

*Average percentage degraded based on 100% present at zero-time control ±SD, $p<0.05$. (5°C x 25°C, sheltered from light ; 5 °C x 25°C, in presence of light; 25°C, sheltered from light x 25°C, in presence of light).

Significant variations in the concentration of the amino acids were observed in the samples removed from the solutions at intervals of 12 hours, 36 hours, 72 hours and 7 days of study.

Comparing the samples kept in the dark at 25°C with those of the same composition stored at 5°C, it was observed a decrease in the concentration of some amino acids after 28 days of storage. Arginine suplemented samples presented degradation greater than 10% in the threonine, methionine, isoleucine, leucine, lysine and proline contents ($p<0.05$).

Degradation smaller than 8% of the same group of amino acids ($p<0.05$) were observed in samples without arginine supplementation, kept in the dark at 25°C. The exception was of the aminoacid lysine which suffered degradation smaller than 5%. Samples A and B, exposed to constant fluorescent light, presented a statistically significant decrease in the content of amino acids in the 28th day ($p<0.05$). No significant change was observed in the compositions of amino acids in samples C, D and E. Analysis of sample F showed no amino acid as expected.

A small decrease in the pH of the solutions a through f occurred at the 28th day of observation (Table 5).

Accentuated changes in the color of the samples A and B stored at 25°C were observed. They ranged from colorless to yellow gold at the 28th day of observation, when compared to the colors of samples A and B at the zero-time control (12 hours) stored at the ideal temperature (5°C).

Microorganisms were not found at intervals of both zero-time and 28 days.

TABLE 5
Average pH of solutions*

	pH of samples (25°C, sheltered from light) A	B	C	D	E	F
Zero-time	7.6	6.1	6.3	8.1	10.7	6.4
28 days	7.3	5.8	6.1	8.0	10.6	6.3

*Determined with pHmeter Sentron model 2001. Based on duplicate samples of each solution.

DISCUSSION

The degradation of the amino acids and the darkening of the parenteral nutrition solutions in samples with and without arginine suplementation (A and B) was probably caused by the Maillard Reaction (8). The Maillard Reaction is a complex sequence of reactions. The first stage of the Maillard Reaction is the condensation of the carbonyl function of glucose with the α-amino group of amino acids. The resulting Schiff base cyclize the glucose to the corresponding glucosylamine which then undergoes an Amadori rearrangement. It follows the decomposition of Amadori and Heyns products leading to

the formation of deoxydicarbonyl sugars. A third and final stage would be the formation of polymers (9, 10).

The Maillard Reaction is undesirable because, besides facilitating the degradation of amino acids, it presents an inhibitory effect in the absorption and metabolism of amino acids and sugars (11). After the formation of lysine glycosides derivatives (12), lysine absortion is inhibited and the metabolism of minerals is decreased (11).

The observation of samples A and B showed a discrete decrease of pH (Table 5). A possible reaction mechanism for this phenomenon is that during the first stage in the formation of the glycosylamine, one or more hydroxy groups of glucose are substituted by an amino group, liberating protons to the reaction environment, thereby contributing to the decrease in the pH of the solution (12).

The heightening in color observed in samples A and B kept at 25°C and in the presence of light during the 28 days of study was probably due to the fact that light can act as a catalyst, accelerating the Maillard reaction in the presence of oxygen (13) when compared to the same samples sheltered from light.

It was found that the concentrations of threonine, methionine, isoleucine, leucine and proline were decreased ($p<0.05$) in sample with arginine-suplemented (A) submitted to 25°C. Analyzing the chemical nature of the degraded amino acids, it can be observed that, with the exception of threonine, which has a polar side chain, the amino acids methionine, isoleucine, leucine and proline have hydrophobic side chains (13). These amino acids are less soluble in water than those with polar side chains and also they are present in an ionized form in pH 6.0-7.0 (the predominant pH in our study), therefore being more ready to react with glucose through the Maillard Reaction.

The lysine, a basic amino acid which presents a positive charge in the position ε of its aliphatic chain, possibly had its degradation accelerated in sample with arginine-supplemented (A) at the 28th day of observation, because of a more favorable pH for the nucleophilic attack on the carbonyl group of glucose (Table 5).

The results also suggest that parenteral nutrition solutions with and without arginine supplemented can be conditioned by a period of 28 days, the temperature of 5°C, without significant alteration in the content of the amino acids.

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