

Arginine effects on survival of young rats with solid Walker 256 tumor

Efeitos da arginina na sobrevivência de ratos jovens com tumor sólido de Walker 256

Efectos de la arginina en la supervivencia de ratones juvenes com tumor sólido de Walker 256

Fabiani Lage Rodrigues Beal¹

Maria Rita Garbi Novaes²

Key words

Arginine; abdominal neoplasms; survival

Unitermos

Arginina; neoplasias abdominais; sobrevivência

Unitérminos

Arginina; neoplasias abdominales; supervivencia

Endereço para correspondência:

Maria Rita Carvalho Garbi Novaes
SHIS QI 09 – Conjunto 6 – Casa 14 – Lago Sul
CEP 71625-060 – Brasília/DF
E-mail: ritanovaes@ig.com.br

Submissão

18 de dezembro de 2006

Aceito para publicação

12 de junho de 2007

Abstract

Arginine supplementation in patients with cancer has been studied in recent years. Presently it is known that supplementation with this amino acid has improved immunologic, biochemical and hematological response, reducing the incidence of metastases besides incrementing nutritional condition in experimental studies with animals and humans. The objective is to evaluate the effect of L-arginine at 8% on extended survival of young rats with solid Walker Tumor. The study is experimental, randomized, double blind. Rats received four inoculations on the lumbar region of 5×10^6 tumor cells. The animals for this experiment were divided in 3 groups. The first group (placebo) received gavages with water, seven days before the inoculation of Walker 256 tumor. The second group received 8% L-arginine solution of caloric ingestion 48 hours after the tumoral inoculation. The third group received 8% L-arginine solution of total caloric ingestion, initiated seven days before the tumoral inoculation. The third group, supplemented seven days before the tumoral inoculation, presented greater extended survival ($p = 0.0001$) when compared to animals from the other two. Animals supplemented 48 hours after the tumoral inoculation presented greater extended survival ($p = 0.0001$) when compared to the placebo group ($p = 0.0001$). The results suggest that both groups supplemented with 8% L-arginine presented beneficial results in respect to extended survival when compared to the placebo group, and better yet if the amino acid was administered seven days before the tumoral inoculation, indicating that arginine was adequate processing is important to minimize metabolic losses of cancer.

Resumo

A suplementação com arginina nos pacientes com câncer tem sido muito investigada nos últimos anos. Atualmente, estima-se que a suplementação dietética com este aminoácido tem melhorado a resposta imunológica, bioquímica e hematológica, reduzindo a incidência de metástases, além de incrementar o estado nutricional em investigações com animais ou seres humanos. O objetivo é avaliar o efeito da L-arginina a 8% no tempo de sobrevivência de ratos jovens com tumor sólido de Walker 256. O estudo é experimental, randomizado, duplo-cego. Os ratos receberam quatro inoculações (multilobular) na região dor lombar, totalizando 5×10^6 células tumorais por sítio. Os animais foram divididos em três grupos. O primeiro grupo, placebo, recebeu água por gavagem sete dias antes da inoculação tumoral, o segundo, L-arginina a 8%, iniciada quarenta e oito horas após a inoculação tumoral, e o terceiro, L-arginina a 8%, começada sete dias antes da inoculação tumoral. Analisando as curvas de sobrevida dos três grupos pelo método de Kaplan-Meier, obteve-se o seguinte resultado no vigésimo dia: a) a probabilidade de que ratos provenientes do grupo que recebeu arginina 7 dias antes da inoculação do tumor sobrevivam após o vigésimo dia de inoculação foi de 42,5%, $p = 0,0001$; b) os ratos pertencentes ao grupo que recebeu arginina 48 horas depois da inoculação apresentaram uma porcentagem de 27,5% em sua sobrevida após o vigésimo dia de inoculação, $p = 0,0001$; c) nos animais do grupo placebo, a probabilidade de sobrevivência após o vigésimo dia foi nula ($p = 0,0001$). Os resultados sugerem que o grupo que recebeu a suplementação com L-arginina a 8%, 48 horas após a inoculação tumoral, apresentou resultados benéficos. Porém, aquele que recebeu a arginina no período de sete dias antes da inoculação tumoral foi o que obteve maior tempo de sobrevivência, sugerindo que ela possa exercer importante função para minimizar as perdas metabólicas do câncer.

Resumen

La suplementación con arginina en los enfermos con cáncer ha sido muy investigada en los últimos años. Actualmente, se estima que la suplementación dietética con ese aminoácido ha

¹ Nutrition Institute. Catholic University, Brasília, Brazil

² Professor, School of Medicine. ESCS/FEPECS/GDF. Professor, Human Nutrition Institute. University of Brasília, Brazil

mejorado la respuesta inmunológica, bioquímica y hematológica, disminuyendo la incidencia de metástasis, además de aumentar el estado nutricional en investigaciones con animales o seres humanos. El objetivo es evaluar el efecto de L-Arginina a 8% en el tiempo de supervivencia de ratones jóvenes inoculados con tumor sólido de Walter 256. Fueron alocados ratones jóvenes inoculados con tumor sólido de Walter 256, siguiendo técnica multilobular en la región dorso-lumbar, con cuatro sitios de inoculación (5×10^6 células tumorales por sitio), en tres grupos experimentales. El primer grupo, placebo, recibió agua por gavage siete días antes de la inoculación tumoral, el segundo grupo, recibió L-arginina a 8% iniciada cuarenta y ocho días después de la inoculación tumoral y el tercer grupo recibió L-arginina a 8% iniciada siete días antes de la inoculación tumoral. Resultados. Analizándose las curvas del tiempo de supervivencia de los tres grupos, por el método de Kaplan-Meier, se obtuvo el siguiente resultado, después del vigésimo día: a) la probabilidad de que los ratones provenientes del grupo que recibió arginina siete días antes más de los veinte días fue de 42,5%, $p = 0,0001$; b) Los ratones que pertenecen al grupo que recibió arginina 48 horas después de la inoculación presentaron un porcentaje de 27,5% en el tiempo de supervivencia después del vigésimo día de la inoculación, $p = 0,0001$; c) En los animales del grupo placebo, la probabilidad de supervivencia después del vigésimo día sería nula ($p = 0,0001$). Los resultados sugieren que el grupo de animales que recibió arginina en el periodo de siete días antes de la inoculación presentó mayor tiempo de supervivencia cuando comparado a los demás grupos, sugiriendo que la arginina pueda ejercer importante función para disminuir las pérdidas metabólicas del cáncer.

Introduction

Many investigations are underway to prevent cancer, increase life expectancy and improve the patient's quality of life¹⁻⁴. The pursuit of these goals has stimulated the search for new drugs and supported traditional and non-traditional pharmacological therapies with certain nutrients, among which Arginine, for its known influence on the immunological system. These nutrients seem to stimulate the production of T lymphocytes, cytokines, nitric oxide, and polyamines. Studies report that dietary supplementation with Arginine in adult cancer patients may have positive effects through a decrease of tumoral growth and extent of life expectancy⁵.

Over the last years many studies have been carried out with the intention of defining one or more substances that are proved to perform this function. Revised studies suggest that dietary arginine supplementation in adult cancer patients presents possible effects on the immunologic system, mainly concerning the alteration of tumoral growth and life expectancy⁶⁻⁸.

There are controversies concerning the pharmacological effect of nutritional supplementation with arginine in the immunologic system of cancer bearers. In clinical and experimental studies, arginine has increased immunity through the association of several mechanisms: release of growth hormone, stimulation of nitric oxide, hydroxyproline, cytokines and polyamines. The regulatory mechanism of the metabolism of this amino acid in tumoral tissues has fundamental importance to evaluate therapeutic elements that effectively prevent tumor genesis⁹⁻¹⁴.

The objective of this work is to evaluate the effect of L-arginine at 8% on the extended survival of young rats, when administered 7 days before and 48 hours after the inoculation of solid Walker 256 tumor.

Methods and materials

Experimental design

The study was carried out with double-blind, placebo-controlled, with random sampling. Male young rats ($n = 60$)

were divided into three groups (placebo, 8% arginine 48 hours after tumoral inoculation and 8% arginine seven days before tumoral inoculation). All groups were submitted to the same intervention, with solution administered every 12 hours, by esophageal gavages, initiated 12 hours after the inoculation, kept until life was interrupted. The experimental group received arginine (arginine cloridrate, Laboratory Ajinomoto Brazil) at 8% dosage of the total amount of calories estimated in a young rat by gavages, in two daily administrations, until their death. The placebo group received a solution without arginine. The start point for counting down the survival of the animals was immediately after the tumoral inoculation until death. The project has been approved by the Ethics Committee in Animals Studies of the University of Brasília, Brazil, and the protocol of the General United Kingdom Coordinating Committee on Cancer Research was followed.

Preparation of animals

Rats Wistar ($n = 60$), males, isogenic, age ranging from 40 to 50 days, were kept under identical temperature, artificial light exposure for 12 hours a day in alternated cycles, and identical amount of food and water (Labina-Purina, Brazil) *ad libitum*.

Inoculated tumor

The Walker 256 tumor (W256 tumor) was kindly provided by the Department of Physiology, IB/UNICAMP. The line originally came from the National Cancer Institute Bank, Cambridge, MA, USA. The tumor is currently kept under liquid N₂ and is maintained through intraperitoneal passages in rats. Rats received four inoculations on the lumbar region of 5×10^6 tumor cells each in 0.25 ml of Ringer-lactate.

Statistical analysis

Data was analyzed with software SAS (Statistical Analysis System), using variance analysis procedures (ANOVA) and Student's test – and later with Duncan's and Student - Newman - Keules tests. Statistically $p \leq 0.05$ was considered significant.

Results

Effects on the survival of animals supplemented with L-arginine

Table 1 shows the results in time of the survival of each group of animals after the tumoral inoculation, starting the day animals begin to dye.

The Table demonstrates that animals' belonging to the placebo group, on the 1^o day of inoculation the probability of survival is 100%; on the 17^o after the inoculation this probability drops to 80%; on the 18^o day it drops to 37.5%; on the 19^o the probability is 22, 5%; and on day 20 no animals from this group will be alive.

It was also observed that animals belonging to the group supplemented 48 hours after the tumoral inoculation, on day one of the inoculation the probability of survival is 100%; on the 19^o day after the inoculation this probability is of 75%; on the 20^o day it drops to 27.5%; on day 21 the probability is that no animals of this group will be alive.

As for animals from the group supplemented seven days before the tumoral inoculation, around day 20 after the inoculation, 42.5% of them have the probability of being alive. The probability drops to 20% on day 21^o; and on day 22^o after the inoculation no animals from this group will be alive.

Analyzing the survival of the three groups through the Kaplan-Meir curve, we found the following results regarding the survival of rats studied after day 20th:

- a) the probability that rats from the group supplemented seven days before the inoculation the survival beyond this period is 42.5%;
- b) rats belonging to the group supplemented 48hs after the inoculation presented 27.5% survival percentage after the 20th day of inoculation; and
- c) a rat belonging to the placebo group, the probability of survival after 20 days is zero.

Comparing the curves of survival (Table 1), we see that rats of the placebo group tend to live less then the group supplemented 48 hours after inoculation, this one tend to live less then the group supplemented seven days before the tumoral inoculation (p = 0.0001).

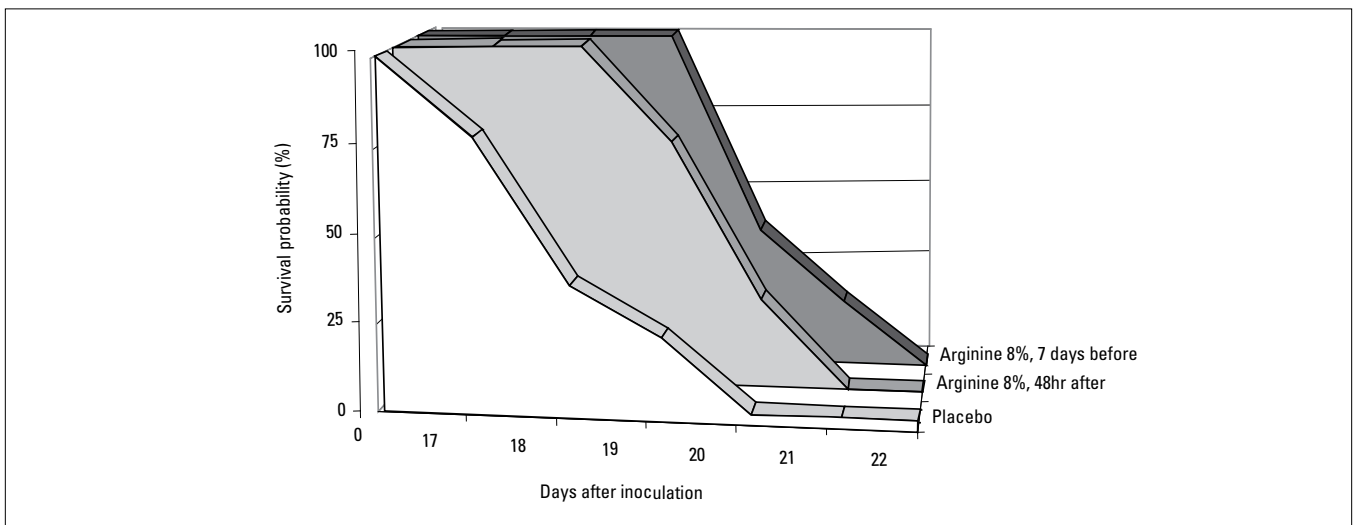
Discussion

The present study has demonstrated a positive and significant correlation among the groups supplemented with L-arginine at 8% of VCT when compared with the placebo group. Also, among controlled groups there were

Table 1 - Survival probability (%).

Group	Day						
	0	17	18	19	20	21	22
Placebo	100.0	80.0	37.5	22.5	0	0	0
Arginine 8%, 48hr after	100.0	100.0	100.0	75.0	27.5	0	0
Arginine 8%, 7 days before	100.0	100.0	100.0	100.0	42.5	20.0	0

p = 0.0001.



Graphic 1 - Survival probability (p = 0.0001).

significant differences, the greatest survival period being presented by the animals of the group supplemented with L-arginine at 8% of total caloric value seven days before tumoral inoculation.

The use of supplements being able to increase immunologic response in preoperative tests, cancer patients, trauma and sepsis, suggests a positive response from those individuals. Experiments indicate that the administrations of immune-nutrients before and after the surgery, in case of trauma, sepsis or cancer, can induce to better results. The same prognosis has been observed in studies with cancer patients and in studies with radio and chemotherapy¹⁵⁻¹⁹.

The majority of works found in science literature relates that supplementation with L-arginine in preoperative stage

should be ministered between 7 to 10 days before the event to guarantee effective stimulation of the immune system through the production of cytokines and polyamines²⁰⁻²².

The collected data in this study corroborates with findings in the literature which demonstrate that when supplementation with L-arginine occurs around the 7th day before the tumoral inoculation, there is a better immune response of the subject under aggression that may be tumoral, trauma or sepsis, such response being a longer survival with less complications²³⁻²⁴.

The findings leads us to believe that supplementation with arginine at 8% beginning seven days before and 48 hours after the tumoral inoculation, have presented beneficial effects on the survival of animals in the controlled groups compared to the placebo group.

Referências bibliográficas

1. Novaes MRG, Beal FLR. Pharmacology of L-arginine in cancer patients. *Rev Bras Cancerol.* 2004;50(4):321-5.
2. Novaes MRG, Pantaleão C. Pharmacological effects of nutritional supplementation of arginine in gastrointestinal cancer patients. *Brazilian Journal of Clinical Nutrition.* 2004; 19(1):26-31.
3. Organización Panamericana De La Salud, Programa Especial de Análisis de Salud. Iniciativa Regional de Datos Básicos en Salud; Sistema de Información Técnica en Salud. Washington DC, 2001. Available in: <<http://www.paho.org/Spanish/sha/coredata/tabulador/newTabulador.htm>> Access in: feb. 2004.
4. Novaes MRG, Lima LAM. Effect of dietetic supplementation with L-arginine in cancer patients. A review of the literature. *Arch Latinoamer Nutr.* 1999;49:301-6.
5. Novaes MRG et al. Metabolic and hemathological effects of dietary supplementation with arginine on rats bearing ascitic Walker 256 tumor. *Ann Nutr Metab.* 2004;48:404-8.
6. Poulin, E. Prophylactic nutrition. *Can J Surg.* 34(6):555-9, 1991.
7. Braga M, Gianotti L, Vignali A, Cestari A, Bisagni P, Di Carlo V. Immunonutrition in gastric cancer surgical patients. *Nutrition.* 1998;14:831-5.
8. Atkinson F, Sieffert E, Bihari D. A prospective, randomised, double-blind, controlled clinical trial of enteral immunonutrition in the critically ill. *Crit Care Medicine.* 1998;26(7):1164-72.
9. Mainous MR, Deitch E. Nutrition and infection. *Surg. Clin. North Am.* 1994;74(3):659-76.
10. Saunders C, Nishikama R, Wolfe B. Surgical nutrition: a review. *J. R. Coll. Surg. Edin.* 1993;38(4):195-204.
11. Brennan MF. Malnutrition in patients with gastrointestinal malignancy. Significance and management. *Dig. Dis. Sci.* 1986; 31:77S-99S.
12. Novaes MRG, Lima LAM, Sousa MV. Maillard's reaction in parenteral solutions supplemented with arginine. *Arch Latinoamer Nutr.* 2001;51:265-8.
13. Novaes MRG, Pantaleão CM. Arginine biochemistry, physiology, and therapeutic implications in gastrointestinal cancer patients. *Rev Cienc Med.* 2005;14(1):67-77.
14. Novaes MRG, Lima LAM, Ribeiro JEG, Magalhães AV, Sousa MV, Morhy L. Pharmacological effects of arginine supplementation in ats with Walker 256 solid tumor. *Arch Latinoamer Nutr.* 2000;50:230-6.
15. De Luis DA, Arranz M, Aller R, Izaola O, Cuellar L, Terroba MC. Immunoenhanced enteral nutrition, effect on inflammatory markers in head and neck cancer patients. *Eur J Clin Nutr.* 2005; 59(1):145-7.
16. Barbul A. Arginine: biochemistry, physiology and therapeutic implications. *J. Parenter. Enteral Nutr.* 1986;10:227-38.
17. Gianotti L, Braga M, Nespoli L, Radaelli G, Beneduce A, Di Carlo V. A randomized controlled trial of preoperative oral supplementation with a specialized diet in patients with gastrointestinal cancer. *Gastroenterology.* 2002;122 (7):1763-70.
18. Heslin MJ, Brennan MF. Advances in perioperative nutrition: cancer. *World J Surg.* 2000;24(12):1477-85.
19. Heyland DK, Novak F, Drove JW, Jain M, Su X, Suchner U. Should immunonutrition become routine in critically ill patients? A systematic review of evidence. *JAMA.* 2001;286 (8):944-53.
20. Van Bokhorst-De Van Der Schueren MA, Quak JJ, von Blomberg-van der Flier BM, Kuik DJ, Langendoen SI, Snow GB, Green CJ, van Leeuwen PA. Effect of perioperative nutrition, with and without arginine supplementation, on nutritional status, immune function, postoperative morbidity, and survival in severely malnourished head and neck cancer patients. *Am J Clin Nutr.* 2001;73(2): 323-32.
21. Tachibana L, Mukai K, Moriguchi S, Takama S, Kishino Y. Evaluation of the effect of arginine-enriched amino acid solution on tumor growth. *J.Parenter.Enteral Nutr.* 1985;9:428-34.
22. Webster NR; Galley HF. Nutrition in the critically ill patient. *J R Coll Surg Edimb.* 2000;45(6):373-9.
23. Reynolds JV, Daly JM, Shou J, Sigal R, Ziegler MM, Naji A. Immunological effects of arginine supplementation in tumor bearing and non tumor bearing hosts. *Ann Surg.* 1990; 211:202-10.
24. Nitenberg G, Raynard B. Nutritional support of the cancer patients: issues and dilemmas. *Crit Rew Oncol Hematol.* 2000; 34(2):137-68.