Walker 256 tümörlü ratlarda Argininle takviye edilen diyetin hayatta kalmaya etkisi

Survival of rats with walker 256 tumor after oral supplementation of Arginine in the diet

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ÖZET

Amaç: Bu çalışma Walker 256 tümörlü ratların %8'lik arginin içeren diyetle sağ kalım sürelerinin arttırılmasını değerlendirmek amacıyla yapılmıştır.

Yöntem: Bu çalışma deneysel, rastgele ve çift kör metodla yapılmıştır. Deneyde ratlar 3 gruba ayrılmıştır. Birinci gruba (placebo) walker 256 tümör aşılanmasından 7 gün önce sonda yardımı ile su verilmiştir. İkinci grup tümör aşılamasından 48 saat sonra %8'lik arginin solüsyonu almıştır. Üçüncü grup ise tümör aşılamasından 7 gün önce %8'lik arginin solüsyonu ile beslenmeye başlanmıştır.

Bulgular: Tümoral aşılamadan 7 gün önce beslenmeye başlayan üçüncü grup diğer iki gruba nazaran daha fazla hayatta kalmıştır. (21 gün p=0.0001) Aşılamadan 48 saat sonra beslenen grupta hayatta kalma süresi (20 gün p=0.0001) placebo grubuna nazaran daha fazladır.

Sonuç: Sonuçlar göstermektedir ki Arginin takviyesi alan iki grup placebo grubuna nazaran hayatta kalma süresi açısından bundan fayda görmüşlerdir. Ayrıca arginin takviyesine tümör aşılamasından önce başlayan grupta bu etki daha fazladır.

Anahtar Kelimeler: Arginin, kanser, hayatta kalım, Walker 256 tümörü.

ABSTRACT

Objective: This study was undertaken to assess the effect of L-arginine at 8% on extended survival of rats with Walker 256 tumor.

Method: The study is experimental, randomized, double blind. The animals for this experiment were divided in 3 groups. The first group (placebo) received gavage 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.

Results: The third group, supplemented seven days before the tumoral inoculation, presented greater extended survival (21 days, p=0.0001) when compared to animals from the other two. Animals supplemented 48 hours after the tumoral inoculation presented greater extended survival (20 days, p=0.0001) when compared to the placebo group (19 days, p=0.0001).

Conclusion: 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 before the tumoral inoculation.

Key Words: Arginine, cancer, survival, Walker 256 tumor.

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INTRODUCTION

Many investigations are underway to prevent cancer, increase life expectancy and improve the patient's quality of life (1-4). 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 expectancy (5).

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 expectancy of patients (6-8). 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 therapeutical elements that effectively prevent tumor genesis (9-14).

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 0.25ml 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 $p<0,05$ 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 diet.

The table demonstrates that animals belonging to the placebo group, on the 1st day of inoculation the probability of survival is 100%; on the 17th after the inoculation this probability drops to 80%; on the 18th day it drops to 37,5%; on the 19th 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 19th day after the inoculation this probability is of 75; on the 20th 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; and on day 22 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- For rats 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).

Table 1. Survival probability (%) of rats with Walker 256 tumor with oral supplementation with arginine 8% in the diet

Group	Day						
	0	17	18	19	20	21	22
Placebo	100,0	80,0	37,5	22,5	0,0	0,0	0,0
Arginine 8%, 48hr after	100,0	100,0	100,0	75,0	27,5	0,0	0,0
Arginine 8%, 7 days before	100,0	100,0	100,0	100,0	42,5	20,0	0,0

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 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 sepses, suggests a positive response from those individuals. Experiments indicate that the administrations of immune-nutrients before and after the surgery, in case of trauma, sepses or cancer, can induce to better results. The same prognosis has been observed in studies with cancer patients and in studies with radio and chemotherapy (15-19).

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 polyamines (20-22). 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 sepses, such response being a longer survival with less

complications (23-24). 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.

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