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Effect of glutamine ingestion on the progression of induced periodontitis: experimental study in rats

Efeito da ingestão de glutamina na progressão de periodontite induzida: estudo experimental em ratos

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Resumo

Introdução: Com a suplementação de glutamina há um melhor desempenho no sistema de defesa do corpo. **Objetivo:** O objetivo deste estudo foi analisar o efeito da suplementação de glutamina na doença periodontal induzida por ligatura em ratos. **Material e método:** Foram selecionados 48 ratos machos da linhagem Wistar divididos em 4 grupos (N = 12): grupo controle (GC) incluindo animais saudaveis recebendo solução salina diariamente via gavagem; grupo glutamina (GG) incluindo animais saudaveis que receberam suplementação oral de glutamina por gavagem a uma dose de 1,5 g/kg/dia; grupo periodontite (GP) incluindo animais com doença periodontal induzida na maxila em ambos os lados recebendo solução salina diária via gavagem; grupo experimental (GE) incluindo animais com doença periodontal induzida na maxila nos dois lados recebendo glutamina diária via gavagem na dose de 1,5 g/kg/dia. No 30º dia, todos os animais foram eutanasiados por excesso de anestésico. **Resultado:** Nas várias análises, o GE (116,63 ± 22,50 mm²) comparado ao GC (82,32 ± 7,48 mm²) obteve um p<0,05. O GP (143,15 ± 35,24 mm²) comparado ao grupo GE (116,63 ± 22,50 mm²) apresentou p<0,05. O GP (143,15 ± 35,24 mm²) comparado ao GC (82,32 ± 7,48 mm²) apresentou p<0,05. O GG (118,09 ± 10,4 mm²) comparado ao GC (82,32 ± 7,48 mm²) apresentou p>0,05. **Conclusão:** A suplementação de glutamina associada à doença periodontal induzida demonstrou uma menor perda óssea em comparação com o grupo periodontite.

Descritores: Suplementos nutricionais; doenças periodontais; produtos com ação antimicrobiana.

Abstract

Introduction: With glutamine supplementation there is better performance in the body's defense system. **Objective:** The aim of this study was to analyze the effect of glutamine supplementation on ligand-induced periodontal disease in rats. **Material and method:** 48 selected male Wistar rats were divided into 4 groups (N = 12): control group (CG) including healthy animals receiving daily saline solution via gavage; glutamine group (GG) including healthy animals receiving oral glutamine supplementation by gavage at a dose of 1.5 g / kg / day; periodontitis group (PG) including animals with induced periodontal disease on both upper sides receiving daily saline solution via gavage; experimental group (EG) including animals with induced periodontal disease on both upper sides receiving daily glutamine via gavage at a dose of 1.5 g / kg / day. On the 30th day, all animals were euthanized by excess anesthetic. **Result:** In the various analyses, the EG (116.63 ± 22.50 mm²) compared to the CG (82.32 ± 7.48 mm²) obtained a p<0.05. The PG (143.15 ± 35.24 mm²) compared to the EG group (116.63 ± 22.50 mm²) presented a p<0.05. The PG (143.15 ± 35.24 mm²) compared to the CG (82.32 ± 7.48 mm²) presented a p<0.05. The GG (118.09 ± 10.4 mm²) compared to the CG (82.32 ± 7.48 mm²) presented a p>0.05. **Conclusion:** Glutamine supplementation associated with induced periodontal disease demonstrated a smaller amount of bone loss compared to the periodontitis group.

Descriptors: Dietary supplements; periodontal diseases; products with antimicrobial action.

INTRODUCTION

Glutamine is the most abundant amino acid in the human body, involving approximately 20% of the total amino acids[1]. Its characteristics are not essential, and there are almost no existing pathologies with glutamine disturbances. Specifically, only three cases have been reported globally[2,3]. Its metabolism is linked to the ammonia cycle[2,3], which is relevant and poses risks to human health.

Glutamine use was evaluated in a clinical trial with children in severe disease states. The results demonstrated that it has a beneficial effect on brain activity and the decrease of ammonia in the body[4]. Furthermore, the findings indicated that glutamine is systemically safe and could be administered in different ways[4,5].

Glutamine plays an essential role in the body's defense system. The decrease in this amino acid causes a drop in the proliferation of lymphocytes and antibodies in response to antigenic stimulation. Thus, in adverse organic conditions, supplementation with glutamine contributes to a lower infection rate and a shorter hospital stay[5,6].

Diseases that affect the periodontium are usually infectious processes[7] that lead to inflammation that manifests clinically between the tooth and the gingiva through the internal epithelium of the gingival sulcus injury. A clear sign of disease activity is the presence of gingival bleeding, which sometimes presents with pain and purulent secretions[8].

Diseases affecting the periodontium are more prevalent in developing countries. However, they are also prevalent in developed countries. Periodontal disease is related to other pathologies including heart disease, smoking, diabetes, and rheumatism. In summary, diseases that affect the periodontium area global public health problem[9,10].

In view of the following, it was expected that a beneficial effect of glutamine supplementation on induced periodontal disease in rats would be found.

MATERIAL AND METHOD

All procedures were performed only after approval from the research ethics committee of the University of Cuiabá (CEP / UNIC), under registration number 050 / CEP / UNIC and protocol number 2012-050.

For the present experiment, 48 rats of the Rattus norvegicus-Wistar strain with a body mass of 349 ± 54 g were selected from the Cuiabá University Laboratory (UNIC). They were provided with a balanced diet (Nuvilab® - Nuvital Nutrientes S / A, Curitiba, PR, Brazil) and water ad libitum, under a light / dark cycle of 12 hours, controlled temperature of 23 °C and humidity of ± 50%. They adapted to the new environment for 7 days.

After the adaptation period, the animals were randomly divided into four distinct groups (N = 12): control group (CG), glutamine group (GG), periodontitis group (PG), and experimental group (EG). The groups with disease induction were induced on both sides of the maxilla.

The control group included healthy animals that received daily saline solution via gavage. The glutamine group included healthy animals that received oral glutamine supplementation via gavage at a dose of 1.5 g / kg / day. The periodontitis group included animals with induced periodontal disease on both upper sides that received daily saline solution via gavage. The experimental group included animals with induced periodontal disease on both upper sides that received daily glutamine via gavage at a dose of 1.5 g / kg / day.

On the first day, all animals were anesthetized with an intramuscular administration of 0.1 mL of ketamine hydrochloride (Dopalen, Agribrands, Animal Health, Paulínia, SP, Brazil) at a dose of 50 mg / mL, which was associated with 0.05 mL of xilazine (Rompun, Bayer, Animal Health, São Paulo, SP, Brazil) and 2 g per 100 mL for each 100 g of body weight. For the animals in the PG and EG groups, a small dislocation was made in the upper second molars (right and left), and a silk suture was placed around the tooth to induce periodontal disease; this procedure was performed in the upper two quadrants, and the silk thread was left in for 30 days. For the animals in the CG and GG groups, only a small dislocation was done in the second molars. All procedures were performed by the same operator who was previously trained.

One day after the surgical procedure, dietary supplementation with glutamine was initiated for the animals in the GG and EG groups via gavage at a single dose of 1.5 g / kg / day in a 50% suspension. With the help of a syringe and a cannula, the animals were immobilized, and all contents were injected into the oral cavity slowly to avoid trauma.

The animals in the CG and PG groups received distilled water during this period at the same volume and equal administration as the EG and GG animals.

All animals were sacrificed on the 30th day of the experiment using excess anesthetic. After euthanasia via excess anesthetic, the maxilla was removed and fixed in 10% formalin.

Body Mass

The animals were weighed before beginning the experiment, after fifteen days of onset, and on the day of euthanasia. An electronic weighing scale was used for the animals, which was regulated according to INMETRO (National Institute of Metrology, Quality and Technology) standards.

Morphometric Analysis

For the morphometric analysis, the pieces were immersed in 30% hydrogen peroxide for two hours, and the soft tissues were removed with gauze, followed by staining with 1% blue methylene for 30 minutes and washing in tap water to remove excess dye. The pieces were dried, and images were taken using a high-resolution camera (Nikon 5100-Thailand, Macro100 - China) with a flash (Sigma, Ronkonkoma-NY, USA). The analyzed parameter was the area in mm^2 of bone loss between the cementum-enamel junction and the alveolar bone crest in the vestibular region, which were the elements in which the disease had been induced; the measurements were assessed using ImageLab software (Diracon Bio Informatics Ltda. Vargem Grande do Sul, SP, Brazil).

Statistical Analysis

According to the analyzed data, the tests were considered appropriate, and constant observation of the condition of homogeneity and normality among the variances using the Shapiro-Wilk test was proposed. For body mass data, analysis of variance (ANOVA) with Tukey's post hoc test was used. For morphometric data, the two-way ANOVA was performed. The confidence interval chosen for the variables was 95%, and the significance level adopted was 5%. The comparisons chosen for this test were as follows:

EG compared to CG; PG compared to EG; PG compared to CG; and GG compared to CG.

RESULT

Regarding the comparisons of the experimental group, animals that used glutamine and animals that used glutamine associated with periodontitis (116.63 ± 22.50 mm²) were compared to the groups in which periodontitis was induced by ligature (143.15 ± 35.24 mm²), and glutamine seemed to be able to decrease the rate of disease progression induced in the periodontium ($p<0.05$). The control group did not receive the medication or have the disease induced by ligature. Thus, the physiological space of the tissues without disease was 82.32 ± 7.48 mm², while in the comparison with the experimental group, it was116.63 ± 22.50 mm² ($p<0.05$) (Table 1).

The animals with ligature-induced periodontitis presented a destruction of the periodontal structures (143.15 ± 35.24 mm²) compared to the control group, which did not receive medication and had no induced disease (82.32 ± 7.48 mm²) ($p<0.05$). The animals in the glutamine group (118.09 ± 10.4 mm²) presented no significant differences compared to the control group (82.32 ± 7.48 mm²) ($p>0.05$).

Table 2 shows the mean body mass of the animals at three different times (initial, 15 days and 30 days), showing no significant differences between the same groups ($p>0.05$). Among the different groups at the same times in the experiment, comparisons between groups did not show significant differences ($p>0.05$).

Table 1. Means of the periodontal destruction obtained in the morphometric analysis

Groups	Means	Standard Deviation
Experimental Group	*116.63	22.50
Control Group	*82.32	7.48
Periodontitis Group	#143.15	35.24
Experimental Group	#116.63	22.50
Periodontitis Group	$143.15	35.24
Control Group	$82.32	7.48
Glutamine Group	&118.09	10.4
Control Group	&82.32	7.48

Two-way ANOVA. *, #, $, & Statistical difference between groups ($p<0.05$). The measurements are in square millimeters (mm²) and refer to the destroyed periodontium.

Table 2. Body mass of animals in grams at different times of the experiment

Groups	Initial body mass		D15 body mass		D30 body mass	
	Mean	SD	Mean	SD	Mean	SD
CG	$359.7^{A, a}$	58.6	$376.6^{B, a}$	58.1	$354.1^{C, a}$	56.8
GG	$361.1^{A, b}$	34.9	$349.5^{B, b}$	28.2	$352.1^{C, b}$	29.6
PG	$339.6^{A, c}$	70.1	$339.2^{B, c}$	54.3	$338.5^{C, c}$	60.7
EG	$336.4^{A, d}$	50.9	$358.6^{B, d}$	35.1	$342.9^{C, d}$	33.5

Intergroup comparison: one-way ANOVA with Tukey's post hoc test, uppercase letters in the columns signify a lack of significant differences ($p>0.05$). Intragroup comparison: ANOVA of repeated measures with Tukey's post hoc test, lowercase letters in the lines signify a lack of significant differences ($p>0.05$). CG: control group; GG: glutamine group; PG: periodontitis group; EG: experimental group; D15: 15 days after the beginning of the experiment; D30: 30 days after the beginning of the experiment; SD: standard deviation.

DISCUSSION

The results of the study demonstrate that the use of glutamine without infection does not present significant differences between the groups; when using glutamine associated with the stimulation of periodontitis and immunoinflammatory disease, the progression of the disease could be avoided compared to the group with only induction of the disease.

The findings of this study possibly occurred because glutamine acted to avoid an effect of oxidative stress. This process is able to stimulate the inflammatory response that causes the destruction of the periodontal tissues[11,12]. In addition to the antimicrobial effect[13], another relevant point is the effect of stimulating the immune system through glutamine.

Apoptosis is programmed cell death. Its occurrence with an increased rhythm in the periodontium is a demonstration of the presence of oxidative stress[14]. In the case of the results of the study, it seems that there was reduced apoptosis even in the presence of oxidative stress. In the case of the periodontium, bacteria called periodontopathogens have the capacity to induce apoptosis, which induces a cascade effect and produces interleukins IL6 and FNT[15].

Glutamine supplementation is commonly used in health care[16]. Its use in the form of supplementation is relevant in patients experiencing polytrauma who are hospitalized in the intensive care unit and patients with cancer and other enteral tract disorders. The results confirm in rats that the study of the use of glutamine[17] can be expanded to dentistry. The most interesting finding was the observation that the group with oral supplementation avoided the progression of periodontitis.

This work can initiate a perspective of the treatment of periodontitis with oral or systemic glutamine supplementation. It should be noted that work on mice is relevant but not applicable to humans. Although glutamine does not present side effects, studies should always be conducted with caution and attention to this possibility.

It is also emphasized that rats were used to evaluate the progression of periodontal disease[18]; we opted to use rats in this study because of the similarities to humans in inflammatory responses, low costs of research, easy handling, similarities regarding the anatomy of molars and the periodontium in their surroundings, and the model of drug intake[18]. The method used to induce the disease was through the placement of the silk thread around the molars, which is very reproducible and low-cost[19,20].

It was observed that body mass remained stable. It is important to emphasize that a simple increase in body mass in reverse becomes

a negative factor in the etiopathogenesis of periodontitis. In 24 rats divided into two groups, one group of 11 rats with a regular diet and another group with 13 rats with a diet high in sugar and fat, periodontal disease were induced in all animals, and after 30 days, the analysis showed that obesity could aggravate periodontal disease[21]. Other studies in humans have already confirmed the same hypothesis[20]; however, the mechanism is unclear[20,21].

In this study, the morphometric method was used. There are several works in the literature that validate all these findings[20-22]. In 2007, Fernandes et al.[16] conducted an experimental study to compare a morphometric analysis with an histological analysis to verify if there was a difference between the methods. For the study, 10 Wistar rats were selected and divided into two groups; periodontal disease was induced in all animals and at the end, their jaws were removed. Five of these jaws were used for the histological analysis, and five were used for the morphometric analysis and at the study's results, the authors did not find statistical difference compared to the measurements made in both methods. The study's conclusion both methods had items that were of value and enabled good information.

It is important to return to the focus of the research question that the described supplementation has little interference with body mass, as the results of this study indicate, but it modulates the inflammatory and oxidative process, favoring the recovery of the organism in the periodontium.

To evaluate the effects on plasma antioxidant protection and renal and pulmonary tissue damage of oral glutamine preceding renal ischemia / reperfusion, 33 rats were selected and submitted to right nephrectomy and divided into three groups: glutamine, control and sham. Fourteen days later, left ischemia and reperfusion were performed. At the end of the experiment, it was concluded that glutamine improves levels of total antioxidant capacity[23,24]. In the results of the described study, it was observed that glutamine showed the potential to avoid bone progression through supplementation of the drug.

In a meta-analysis conducted in 2014, Liu et al.[25], sought to seek evidence of the association of chronic periodontal disease with biomarkers of oxidative stress. Of 329 articles evaluated, 16 were selected because they had great scientific impact. The authors concluded that total antioxidant capacity decreased in the presence of chronic periodontitis and that levels of malondialdehyde and nitric oxide were higher compared to healthy patients.

This study seems to agree with the other papers presented here. Glutamine decreased bone loss caused by induced periodontitis, which was evaluated in the morphometric analysis. However, the relationship between glutamine and periodontal disease needs to be explored further since the literature lacks information. These results are expected to be used to support further research and perhaps contribute to a clinical trial on glutamine use.

CONCLUSION

Glutamine associated with the induction of periodontitis demonstrated less bone loss compared with the group with only periodontitis.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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