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ORIGINAL ARTICLE

Glutamine and alanyl-glutamine accelerate the recovery from 5-fluorouracil-induced experimental oral mucositis in hamster

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Abstract
Introduction Mucositis induced by anti-neoplastic drugs is an important, dose-limiting and costly side effect of cancer therapy.
Aim To evaluate the effect of oral glutamine and alanyl-glutamine, a more stable glutamine derivative, on 5-FU-induced oral mucositis in hamsters.
Materials and methods Oral mucositis was induced by two intraperitoneal (i.p) administrations of 5-FU on the first and second days of the experiment (60 and 40 mg/kg, respectively) followed by mechanical trauma on the fourth day in male hamsters. Animals received saline, glutamine or alanyl-glutamine suspension (100 mM) 1 h before the injections of 5-FU and daily until sacrifice, on the 10th or 14th day. Macroscopic and histopathological analyses were evaluated and graded. Tissues from the cheek pouches were harvested for measurement of myeloperoxidase activity and glutathione stores. For investigation of serum concentration of glutamine, blood was obtained by heart puncture from anesthetized animals before sacrifice, on day 10.
Results Treatment with glutamine and alanyl-glutamine reduced macroscopic and histological parameters of oral mucositis, and reduced the myeloperoxidase activity on day 14, but not on day 10. The 5-FU-induced oral mucositis significantly decreased the serum glutamine levels as well as the cheek pouch glutathione stores observed on day 10. Glutamine or alanyl-glutamine administration reversed the 5-FU effects, restoring serum glutamine levels and cheek pouch glutathione stores, observed on day 10, but did not prevent oral mucositis on the tenth day.
Conclusion Glutamine or alanyl-glutamine accelerated the mucosal recovery increasing mucosal tissue glutathione stores, reducing inflammatory parameters and speeding reepithelization.

Keywords 5-fluorouracil · Oral mucositis · Glutamine · Alanyl-glutamine

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Introduction

Oral mucositis is a frequent and dose-limiting effect of chemotherapy. In patients receiving 5-FU, it has been estimated that as many as 40% may develop oral mucositis [1]. High-risk protocols can produce severe mucositis rates in excess of 60% [2]. This condition is associated with discomfort symptoms, decreasing patients' quality of life, and increasing economic costs as well as risk of infection and sepsis [3].

Historically, mucositis was viewed solely as an epithelium-mediated event, which was the result of the nonspecific toxic effects of radiation or chemotherapy on dividing stem cells [4]. It was believed that direct damage by chemotherapy or radiation therapy to the basal epithelial cell layer led to loss of the renewal capacity of the epithelium,

resulting in clonogenic cell death, atrophy, and consequent ulceration. New research, however, has suggested that mucositis is not just an epithelial process but involves all the tissues of the mucosa, as evidenced by recent data involving morphologic findings, proinflammatory cytokines, platelet aggregation, endothelial and connective tissue injury, and tissue apoptosis [3].

Glutamine is the most abundant free amino acid in the human body and its flux between tissues is greater than that of any other amino acid [5]. It is essential for the growth of normal and neoplastic cells and for the culture of many cells types [6].

Several studies have evaluated the benefit of oral or parenteral glutamine supplementation in cancer patients receiving chemotherapy and/or radiotherapy or after bone marrow transplant, with conflicting results. Confirming a former non-randomized pilot study [7], the same research group published a randomized, double blind, crossover trial in cancer patients receiving chemotherapy which showed that oral glutamine appears to be useful to increase the comfort of patients at high risk of mouth sores as a consequence of chemotherapy [8]. Other studies also show that oral glutamine decreases the severity and duration of oropharyngeal mucositis in patients undergoing bone marrow transplantation [9]. However, there are also reports in which glutamine fails to alleviate oral mucositis in patients from bone marrow transplants and also in patients in use of 5-fluorouracil (5-FU) [10–12]. A recent double blind, placebo-controlled trial, in head and neck cancer patients treated with chemoradiotherapy, demonstrated that intravenous L-alanyl-L-glutamine reduced the number of patients with severe oral mucositis and that those in use of alanyl-glutamine experienced less pain compared to placebo treated group [13].

Most of the studies on the effect of glutamine and alanyl-glutamine on oral mucositis are clinical trials, which restrict investigation to the mechanism. The present study uses a well described animal model of oral mucositis [14–16] to add information on the pathogenesis of 5-FU-induced oral mucositis and on the effect of glutamine and its more stable derivative, alanyl-glutamine, on the course of 5-FU-induced oral mucositis under hitherto unstudied parameters such as neutrophil infiltration, glutamine serum level, glutathione tissue level.

Materials and methods

Animals

Sixty male adult Golden hamsters weighing 140–160 g from the Federal University of Ceará, were housed in temperature-controlled rooms and received water and food ad libitum. Surgical procedures and animal treatments were conducted in accordance with the guidelines for Institutional Animal Care and Use of Federal University of Ceará, Brazil.

Drugs

Glutamine and alanyl-glutamine were purchased from Sigma–Aldrich (St. Louis, MO, USA). The 5-FU used is a product of Roche, Rio de Janeiro, Brazil.

Induction of experimental oral mucositis

Oral mucositis was induced by two intraperitoneal (i.p) administrations of 5-FU on the first and second days of the experiment (60 and 40 mg/kg, respectively), according to an experimental oral mucositis model previously described [14–16]. In order to mimic the friction to which the oral mucosa is normally subjected, the animal cheek pouch mucosa was irritated by superficial scratching with the tip of an 18-gauge needle on the fourth day, under anesthesia with chloral hydrate (250 mg/kg, i.p.). The needle was dragged twice in linear fashion across the everted cheek pouch until erythematous changes were noted. The animals were sacrificed on the 10th or 14th days after the initial injection of 5-FU, under anesthesia with chloral hydrate (250 mg/kg, i.p.). The cheek pouches were everted and photographed and then the hamster was sacrificed. Samples of cheek pouches were removed from 6 animals per group for histopathological analysis, measurement of glutathione stores, and for myeloperoxidase assay. For investigation of serum concentration of glutamine, blood was obtained by heart puncture from anesthetized animals, on day 10, before sacrifice.

Experimental groups

Hamster groups with oral mucositis received oral administration of glutamine or L-alanyl-glutamine (100 mM) 1 h before the injections of 5-FU and daily until sacrifice, on days 10 or 14. Control groups consisted of animals not subjected to oral mucositis (normal), a non-treated group which was subjected to the experimental mucositis by 5-FU administration and mechanical irritation and received oral administration of saline (5-FU), and a group which received only mechanical trauma of cheek pouches on the fourth day (MT).

The serum concentration of glutamine assay

The serum concentration of glutamine was investigated on day 10. Blood was collected by heart puncture from anesthetized animals, using syringes, just before sacrifice. The tubes were centrifuged for 15 min at 3,000*g* at 4°C. Samples

were analyzed for glutamine by reversed-phase high-performance liquid chromatography (HPLC) using ultraviolet detection, previously described [17].

Macroscopic analysis of cheek pouch

Photographs were used for scoring lesions. For macroscopic analysis, inflammatory aspects such as erythema, hyperemia, hemorrhagic areas, epithelial ulcerations and abscesses were evaluated in a single-blind fashion and graded as follows. *Score 0*: normal cheek pouch with erythema and hyperemia absent or discreet; absence of hemorrhagic areas, ulcerations or abscess. *Score 1*: moderate erythema and hyperemia; absence of hemorrhagic areas, ulcerations or abscess. *Score 2*: severe erythema and hyperemia; presence of hemorrhagic areas, small ulcerations or scarred tissue, absence of abscess. *Score 3*: severe erythema and hyperemia; presence of hemorrhagic areas, extensive ulcerations and abscesses.

Histopathological analysis

The specimens were fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. Sections of 5 µm thickness were obtained for hematoxylin–eosin staining (H&E) and examined by light microscopy (×40). The parameters of inflammatory cell infiltration, vascular dilatation and ingurgitation, presence of hemorrhagic areas, edema, ulcerations and abscesses were determined in a single-blind fashion and graded as previously described [15, 16]. *Score 0:* normal epithelium and connective tissue without vasodilatation; absence of or discreet cellular infiltration; absence of hemorrhagic areas, ulcerations or abscesses. *Score 1*: discreet vasodilatation, re-epithelization areas; discreet inflammatory infiltration with mononuclear prevalence; absence of hemorrhagic areas, edema, ulcerations or abscesses. *Score 2*: moderate vasodilatation, areas of hydropic epithelial degeneration, inflammatory infiltration with neutrophil prevalence, presence of hemorrhagic areas, edema and eventual ulcerations, absence of abscesses. *Score 3*: severe vasodilatation, inflammatory infiltration with neutrophil prevalence, presence of hemorrhagic areas, edema and extensive ulceration and abscesses.

Non-protein sulfhydryl group assay

Cheek pouches samples were harvested and stored at −70°C until required for assay. Non-protein sulfhydryl groups (NP-SH) belong to a group of substances, mainly represented by glutathione, that are involved in cytoprotection by preventing free radical oxidative damage in various tissues [18]. NP-SH concentration was measured using a previously described assay [19]. Briefly, 50–100 mg of frozen cheek pouch tissue were homogenized in 1 ml of 0.02 M EDTA for each 100 mg of tissue. Aliquots of 400 µl of the homogenate were mixed with 320 µl distilled H_2O and 80 µl of 50% trichloroacetic acid (TCA) to precipitate proteins. The tubes were centrifuged for 15 min at 3,000*g* at 4°C. A total of 400 µl of supernatant was mixed with 800 µl of 0.4 M Tris buffer, pH 8.9, along with 20 µl DTNB, and the mixture was shaken for 3 min. The absorbance was read within 5 min of the addition of [5, 5′dithiobis-(2-nitro-benzoic acid)] (DTNB, Fluka) at 412 nm against a reagent blank with no homogenate. Tissue NP-SH content was reported as µg/mg of tissue.

Myeloperoxidase assay

Cheek pouch samples were harvested and stored at −70°C until required for assay. After homogenization and centrifugation (4,500*g*, 20 min), activity of myeloperoxidase, an enzyme found in azurophil neutrophil granules, used as a marker for the presence of neutrophils in inflamed tissue, was determined by a colorimetric method described previously [20] and expressed as units of MPO per 5 mg of tissue.

Statistical analysis

Data were described as either means ± SEM or median, as appropriate. Analysis of variance (ANOVA) followed by Bonferroni's test was used to compare means and Kruskal–Wallis and Dunns tests to compare medians; $P < 0.05$ was defined as statistically significant.

Results

Effect of glutamine and alanyl-glutamine macroscopic and histopathological analysis of oral mucosa

The i.p. administration of 5-FU, followed by mechanical trauma of the cheek pouch of the animals caused significant lesions ($P < 0.05$), observed on day 10, represented by accentuated erythema, hemorrhage, extensive ulcers and abscesses (Fig. 1c; Table 1), when compared to the group of normal animals control (Fig. 1a; Table 1) or to animals submitted to mechanical trauma only (MT; Table 1). These 5-FU effects observed on day 10 were not significantly prevented by oral administration of glutamine (100 mM), nor alanyl-glutamine (100 mM) (Table 1). On day 14, treatment with both alanyl-glutamine (Fig. 1g; Table 1) and glutamine (Fig. 1i; Table 1) significantly ($P < 0.05$) reduced inflammatory alterations when compared to the non-treated group which was subjected to the experimental mucositis by 5-FU administration and mechanical irritation

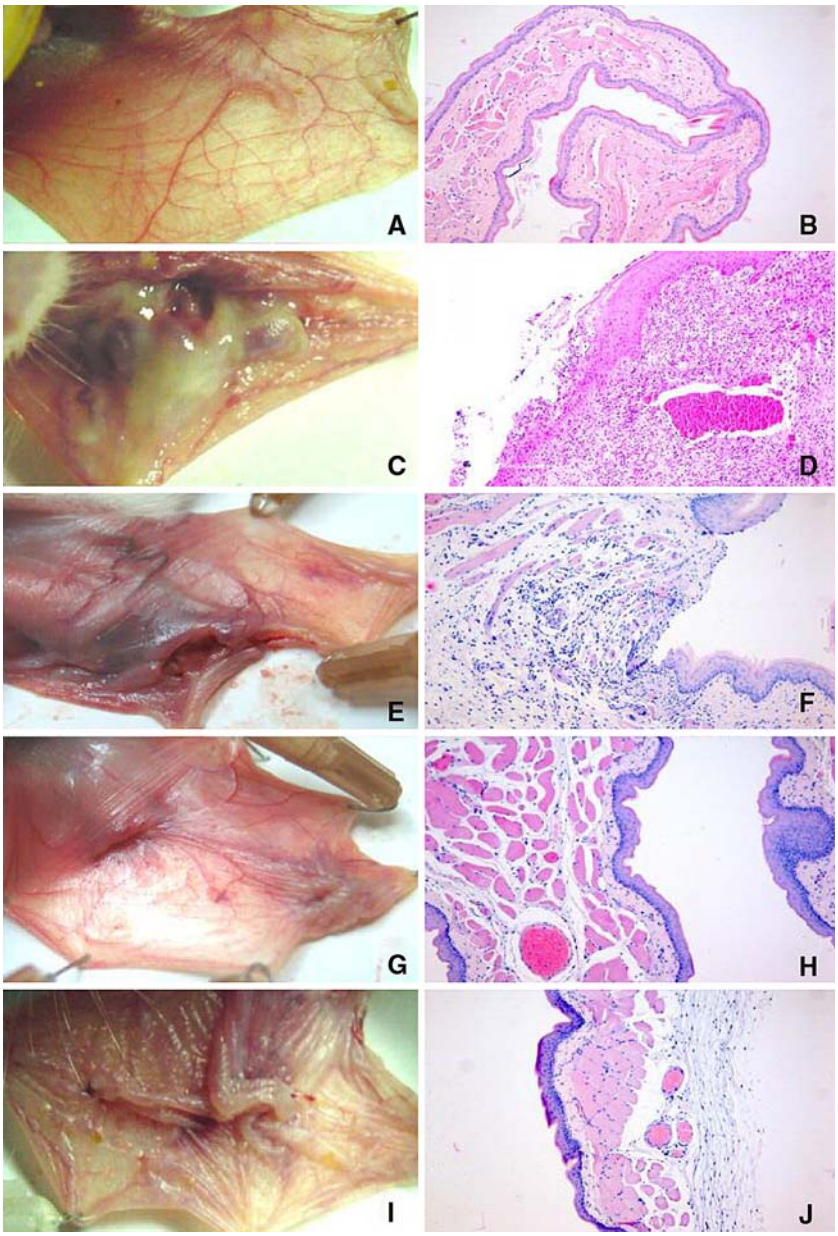

Fig. 1 Macroscopic and microscopic aspects of normal hamster cheek pouches (**a** and **b**) or cheek pouches of animals submitted to 5-fluorouracil (5-FU)-induced oral mucositis, receiving saline, observed on day 10 (**c** and **d**) and on day 14 (**e** and **f**), glutamine (**g** and **h**) or alanyl-glutamine, both observed on day 14 (**i** and **j**). Oral mucositis was induced by intraperitoneal injection of 5-FU followed by mechanical trauma of the cheek pouch. Animals received oral administration of glutamine (100 mM), alanil-glutamine (100 mM) or 0.5 ml of saline, 1 h before 5-FU and daily for 10 or 14 days. Each cheek pouch was everted and photographed, and samples were removed and processed for hematoxylin & eosin staining (×40 magnification) after the animal was killed

Table 1 Effect of glutamine and alanyl-glutamine on macroscopic and microscopic appearance of cheek pouches of hamsters submitted to 5-fluorouracil (5-FU)-induced experimental oral mucositis, observed on days 10 and 14

Experimental groups	Normal	MT	5-FU					
			Saline 10th day	Saline 14th day	GLU 10th day	GLU 14th day	AL-GLU 10th day	AL-GLU 14th day
Macroscopic analysis	0 (0–0)	0.5 (0–1)	3(2–3)*	2(2–3)*	3(1–3)*	2(1–2)*,**	3(1–3)*	2(1–2)*,**
Microscopic analysis	0 (0–0)	0.5 (0–1)	3(1–3)*	2(1–3)*	2(1–3)*	2(1–3)*,**	2(1–3)*	1(1–2)*,**

Oral mucositis was induced in hamsters by intraperitoneal (i.p.) injection of 5-fluorouracil (5-FU) and by mechanical trauma (MT) of the cheek pouch. Animals received glutamine (GLU; 100 mM) alanyl-glutamine (AL-GLU; 100 mM) or 0.4 ml of saline, 1 h before 5-FU and daily for 14 days. Data represent the median values (and range) of macroscopic or microscopic scores in at least 6 animals per group

*$P < 0.05$ compared to normal animals; **$P < 0.05$ compared to animals submitted to 5-FU-induced oral mucositis that received saline, observed on the 14th day. Data were analyzed by using Kruskal–Wallis and Dunn's tests

(5-FU), which still presents erythema and edema but with visible healing process (Fig. 1e; Table 1).

The histopathology of the cheek pouch of animals subjected to 5-FU-induced oral mucositis, observed on day 10, revealed accentuated vasodilatation, intense cellular infiltration with neutrophil prevalence, hemorrhagic areas, edema, abscesses and extensive ulcers (Fig. 1d; Table 1), when compared to the cheek pouches of normal hamsters (Fig. 1b, Table 1) and to the mechanical trauma group (Table 1). Neither treatment with oral glutamine, nor alanyl-glutamine (Table 1) significantly ($P < 0.05$) reduced the 5-FU-induced inflammatory cell infiltration, edema and hemorrhage, nor did it prevent the formation of ulceration and abscesses observed on the 10th day of the experiment. On the other hand, we observed on the 14th day, an increased re-epithelization in the cheek pouches of animals treated with oral L-alanyl-glutamine (Fig. 1h, Table 1) or glutamine (Fig. 1j, Table 1) when compared to the non-treated group (5-FU; Fig. 1f, Table 1).

Effect of 5-FU on serum concentration of glutamine

The i.p. administration of 5-FU significantly ($P < 0.05$) decreased the serum glutamine levels observed on day 10, when compared to the normal control group (Normal). The daily oral administration of glutamine or alanyl-glutamine has restored the serum glutamine levels, measured on day 10 (Fig. 2).

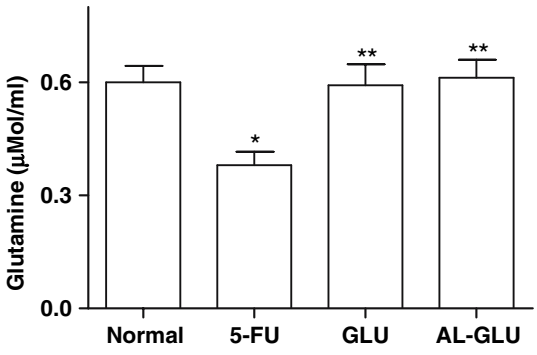

Fig. 2 The 5-fluorouracil (5-FU)-induced oral mucositis decreased the serum glutamine levels measured on day 10. The oral administration of glutamine or alanyl-glutamine has restored the serum glutamine levels. Oral mucositis was induced in hamsters by the intraperitoneal (*i.p.*) injection of 5-fluorouracil (*5-FU*) followed by mechanical trauma (*MT*). Animals received oral administration of glutamine (100 mM), alanyl-glutamine (100 mM) or 0.4 ml of saline, 1 h before each 5-FU injection and daily for 10 days. Before sacrifice, blood was collected by heart puncture from anesthetized animals by using syringes, in order to investigate the serum concentration of glutamine. *Bars* represent the mean value ± standard error of the mean (*SEM*) of the serum glutamine levels (µMol/ml). *$P < 0.05$ represents statistical differences compared to normal group (N); **$P < 0.05$ represents statistical differences compared to non-treated 5-FU-induced oral mucositis group (5-FU). Data were analyzed by using analysis of variance (*ANOVA*) and Bonferroni tests

Effect of glutamine and alanyl-glutamine in non-protein sulfhydryl group assay

The cheek pouch tissue glutathione stores of the animals subject to 5-FU-induced experimental oral mucositis were significantly decreased ($P < 0.05$) when compared to normal group on day 10. The oral administration of both glutamine and alanyl-glutamine significantly ($P < 0.05$) reversed the 5-FU effects, increasing cheek pouch glutathione stores (Fig. 3).

Effect of glutamine and alanyl-glutamine on 5-FU-induced increase in MPO activity in hamster cheek pouch

The myeloperoxidase (MPO) activity on the cheek pouch tissue of animals subject to 10 days of 5-FU-induced experimental oral mucositis was significantly increased ($P < 0.05$) in comparison to normal group or to the group of animals submitted to mechanical trauma only (MT). Neither glutamine nor alanyl-glutamine blocked this elevation in MPO activity in the cheek pouch on day 10. On the 14th day, alanyl-glutamine and glutamine significantly ($P < 0.05$) reduced the 5-FU-induced increase of MPO activity as compared to MPO activity on the 10th day (Fig. 4).

Discussion

In this study, we demonstrated a decrease of serum glutamine levels in the group of hamsters that received 5-FU.

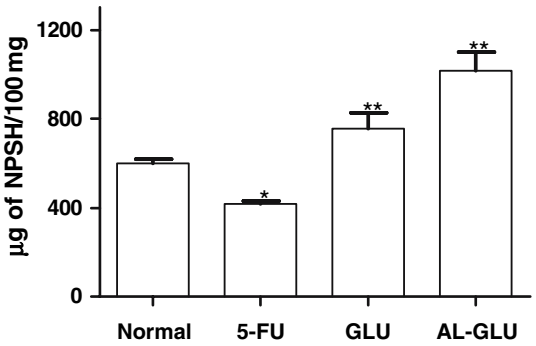

Fig. 3 Glutamine and alanyl-glutamine increased the cheek pouches glutathione stores on the tenth day. Oral mucositis was induced in hamsters by the intraperitoneal (*i.p.*) injection of 5-fluorouracil (*5-FU*) followed by mechanical trauma (*MT*). Animals received oral administration of glutamine (100 mM), alanil-glutamine (100 mM) or 0.4 ml of saline, 1 h before each 5-FU injection and daily for 10. After sacrifice, a sample of the cheek pouch was removed for measurement of glutathione stores. *Bars* represent the mean value ± standard error of the mean (*SEM*) of the stores of glutathione (µg/mg of tissue) of cheek pouch. *$P < 0.05$ represents statistical differences compared to normal control group (N); **$P < 0.05$ represents statistical differences compared to non-treated 5-FU-induced oral mucositis group (5-FU); Data were analyzed by using analysis of variance (*ANOVA*) and Bonferroni tests

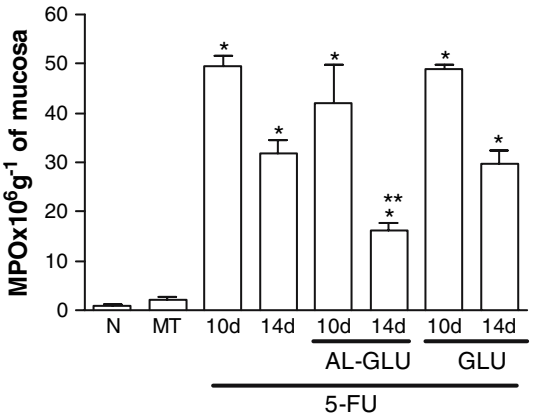

Fig. 4 Glutamine (*GLU*) and alanil-glutamine (*AL-GLU*) inhibit myeloperoxidase (*MPO*) activity in the cheek pouch of hamsters submitted to oral mucositis only on the 14 th. Oral mucositis was induced in hamsters by the intraperitoneal (*i.p.*) injection of 5-fluorouracil (*5-FU*) followed by mechanical trauma (*MT*). Animals received oral administration of glutamine (100 mM), alanil-glutamine (100 mM) or 0.4 ml of saline, 1 h before each 5-FU injection and daily for 10 or 14 days. After sacrifice, a sample of the cheek pouch was removed for MPO activity. Bars represent the mean value ± standard error of the mean (SEM) of the concentration of MPO × $10^6\,g^{-1}$ of cheek pouch. *$P < 0.05$ represents statistical differences compared to normal and mechanical trauma (*MT*) groups; **$P < 0.05$ represents statistical differences compared to non-treated 5-FU-induced oral mucositis group (5-FU 14 days). Data were analyzed by using analysis of variance (*ANOVA*) and Bonferroni tests

Glutamine was classified as a non-essential amino acid, but in more recent years it has been shown that despite a large repository of glutamine, stores may become depleted, particularly in the course of many catabolic assaults such as injury, infection, or chronic glucocorticoid treatment [21]. This appears especially important for susceptible individuals, such as postoperative patients, very low birthweight infants and individuals with cancer [22]. It has been demonstrated that tumor-growth depletes the host glutamine stores, resulting in cachexia [23]. Accordingly, patients with head-and-neck cancer are naturally depleted of glutamine [24], a condition that may be exacerbated by the effects of cancer treatment as suggested by data presented here. The demonstration that 5-FU induced glutamine depletion, prompts us to investigate the role of glutamine or alanyl-glutamine supplementation in the course of 5-FU-induced oral muscositis. In view of this, we would expect glutamine supplementation to have benefits in 5-FU-induced oral mucositis.

Although the reposition of glutamine or alanyl-glutamine, in the dosage used in this study (73 and 108 mg/kg per day, respectively), completely reversed the 5-FU-induced depletion of serum glutamine levels, and also increased the tissue glutathione stores, it did not reduce the lesions found in 5-FU-induced experimental oral mucositis on the tenth day of experiment, corresponding to maximal mucositis in hamsters [15]. All the macroscopic aspects were confirmed by histopathological analysis, revealing, on day 10, severe vasodilatation, accentuated inflammatory infiltration with neutrophil prevalence, hemorrhagic areas, edema, ulcerations and abscess in the cheek pouches of the group of animals submitted to 5-FU-induced oral mucositis that received saline, as well as in the group treated with glutamine or alanyl-glutamine.

This finding could be related to the mechanism of cytotoxicity of the chemotherapy agent used in this study. 5-FU is a competitive inhibitor of thymidylate synthetase with consequent thymidine deficiency resulting in inhibition of deoxyribonucleic acid (DNA) synthesis [25]. In addition, incorporation into ribonucleic acid (RNA) interferes with processing and function of RNA, and has been associated with toxicity [26]. The effects of DNA and RNA deprivation are most significant in cells with a high mitotic index, including the normal, rapidly proliferating tissues of the bone marrow and the lining of the gastrointestinal tract [27]. In view of this, despite the fact that glutamine is essential for cell proliferation, being an important metabolic substrate for rapidly replicating cells [28] it was not capable of antagonizing the effect of 5-FU on the inhibition of cell proliferation 10 days after its first administration.

In accordance with our data, previous clinical trial showed that neither glutamine nor alanyl-glutamine supplementation had any effect on oral mucositis in autologous transplant patients [12], neither on 5-FU and folinic acid-induced mucositis in patients with gastrointestinal cancer [10]. Prophylactic use of glutamine also failed to alleviate 5-FU-induced mucositis [11].

However, our results clearly showed that glutamine and alanyl-glutamine accelerated the healing process, observed on the 14th day after the first administration of 5-FU, suggesting that when 5-FU potent antimetabolic effect declines, glutamine and alanyl-glutamine can exert their proliferative action. The positive effect of glutamine and alanyl-glutamine on the healing phase of 5-FU-induced oral mucositis was associated with reduced neutrophil infiltration detected by histopathology and by the reduced mucosal tissue myeloperoxidase activity. Others have shown accelerated healing of the intestinal mucosa occurring in rodents receiving glutamine-supplemented nutrition and 5-FU [29]. Another study showed that oral glutamine decreased the severity and duration of oropharyngeal mucositis [8]. Studies by our group also demonstrated that glutamine and alanyl-glutamine did not prevent 5-FU intestinal structural damage, but alanyl-glutamine was able to speed intestinal recovery [30]. These data suggest that the conflicting results regarding the effect of glutamine on oral mucositis could be related to differences in the time course of its effect and the timing of evaluation in each study.

Different factors may be taken into account to explain the benefits of exogenous glutamine in hastening oral mucosa healing. First, it has been demonstrated that glutamine can activate ornithine descarboxylase, a first and rate-limiting enzyme in polyamine synthesis in a dose- and time dependent manner, thereby enhancing DNA synthesis. In addition, glutamine can activate mitotic signaling pathways, including mitogen-activated protein kinases and transcription factors, leading to proliferative responses [31, 32]. Second, previous studies have suggested that glutamine augments host defenses and may be important in glutathione synthesis thus decreasing the oxidative stress [33–36]. Accordingly, our data demonstrated that the administration of glutamine as well as alanyl-glutamine increased the mucosal tissue glutathione stores in 5-FU treated hamsters.

Cysteine and other thiol compounds have been considered rate-limiting for glutathione biosynthesis, but it has been demonstrated that glutamine becomes essential during metabolic stress to restore tissue glutathione levels which have become depleted [33, 37, 38].

Glutathione, the major intracellular antioxidant, is involved in several fundamental biological functions, including free radical scavenging, detoxification of xenobiotics and carcinogens, redox reactions, and biosynthesis of DNA and proteins [23], being essential to normal cell function and replication [34]. In addition, glutathione has an inhibitory effect on a number of cytokines [39, 40]. Thus, our result suggests that glutamine bioavailability by exogenous administration, restored glutathione level thereby enhancing cell protection, as well as regulating cell proliferation after exposure to 5-FU that generate toxic quantities of free radicals [3]. On day 5, glutamine and alanyl-glutamine was not able to reversed the effect of 5-FU over the mucosal tissue glutathione stores (data not shown), which could be an additional explanation for the delayed effect of these substances on 5-FU induced mucosal damage.

In conclusion, this study demonstrated that 5-FU significantly decreased serum glutamine levels, as well as cheek pouch glutathione stores. Glutamine or alanyl-glutamine administration reversed the 5-FU effects, restoring the serum glutamine levels and the cheek pouch glutathione stores, but did not prevent oral mucositis on the tenth day. Therefore, glutamine or alanyl-glutamine accelerated mucosal healing with reduction of inflammatory parameters and increased re-epithelization on day 14.

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