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Experimental Work

L-glutamine Supplemented Nutrition Alleviates Damage Caused by Corrosİve Esophagitis in Rats

L-glutamin Destekli Beslenme Ratlarda Koroziv Özefajit Hasarını Azaltmaktadır

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ABSTRACT

Objective: The primary goal in the treatment of corrosive esophagitis (CE) is to control inflammation and scar reactions. L-glutamine (Gln) is beneficial for the integrity of the intestinal mucosal epithelium and is an amino acid that promotes mature collagen growth. This study was designed to demonstrate the positive results of Gln on injury in corrosive esophagitis.

Method: Thirty Sprague-Dawley rats were used in the study. They were divided into 3 groups. CE was formed by dripping 20% sodium hydroxide into the distal esophagus in both groups except the control group (n=10). First group (n=9) was left untreated, while the other group (n=9) was fed orally with the addition of 1 g/kg Gln once a day for 21 days. All rats were sacrificed after 3 weeks. Esophagus of treated and other group rats were examined under light microscope to evaluate collagen deposition, histological damage score and stenosis index.

Results: Excess submucosal collagen, muscularis mucosal damage, inflammation and ulceration, which are among the histological damage score parameters, were significantly higher in the untreated group than in the Gln group (p=0.005, p=0.015, p=0.001, respectively). The stenosis index was significantly different (p=0.013). The group treated with Gln had inflammation but no ulceration and necrosis.

Conclusion: Our experimental animal study suggests that Gln in nutrition reduces damage in the esophageal mucosa, slows down or partially stops the cellular destruction process that causes stenosis,

Keywords: Caustics, corrosives, esophagitis, L-glutamine, esophagial stricture

ÖZ

Amaç: Koroziv özofajit tedavisinde birincil amaç enflamasyon ve skar reaksiyonlarını baskılayarak kontrol etmektir. L-glutamin, bağırsak mukozal epitelinin bütünlüğünü ve matür kollajen büyümesini destekleyen bir amino asittir. Bu çalışma, L-glutamin'in korozif özofajitte oluşan özofagial hasar üzerindeki olumlu sonuçlarını değerlendirmek için tasarlanmıştır.

Yöntem: Çalışmada 30 adet Sprague-Dawley cinsi sıçan kullanıldı. Üç gruba ayrıldılar. Kontrol grubu (n=10) hariç her iki grupta da distal özofagusa %20 NaOH (sodyum hidroksit) damlatılarak korozif özofajit oluşturuldu. Birinci grup (n=9) tedavi edilmeden bırakıldı, diğer grup (n=9) 21 gün boyunca günde bir kez 1 g/kg L-glutamin ilave edilerek ağızdan beslendi. Tüm sıçanlar 3 hafta sonra sakrifiye edildi. Kollajen birikimi, histolojik hasar skoru ve stenoz indeksini değerlendirmek için tedavi edilen ve diğer grup sıçanların yemek borusu ışık mikroskobu altında incelendi.

Bulgular: Histolojik hasar skoru parametrelerinden olan aşırı submukozal kollajen, muskularis mukozal hasar, enflamasyon ve ülserasyon tedavi edilmeyen grupta L-glutamin grubuna göre anlamlı derecede yüksekti (p=0.005, p=0.015, p=0.001, sırasıyla). Stenoz indeksi önemli ölçüde farklıydı. (p=0.013). L-glutamin ile tedavi edilen grupta enflamasyon saptandı ancak ülserasyon ve nekroz görülmedi.

Sonuç: Çalışmamız, L-Glutamin'in deneysel oluşturulmuş koroziv özefajit de ortaya çıkan mukozal hasarı azalttığını, stenoza neden olan hücresel yıkım sürecini yavaşlattığını veya kısmen durdurduğunu histopatolojik bulgularla göstermiştir.

Anahtar kelimeler: Kostik, koroziv, özefajit, L-glutamin, özefagus darlığı

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INTRODUCTION

Corrosive esophagitis (CE) is a devastating health problem in children. Such injuries result in narrowing and even perforation of the digestive tract and more particularly of the esophagus [1]. Mucosal burns are more common in the acute phase and death may occur rarely after perforation. In the long-term, it causes stenosis at the injury site as a late complication [2,3]. The process of corrosive esophageal stricture occurs with reactive inflammation and edema, followed by granulation [4]. There is no consensus on the use of antibiotics and steroids for treatment, and some studies have shown no benefit from their use [5,6].

The primary objective of the medical treatment is to reduce the development of inflammation, fibroplasia, and scar reactions to prevent stricture. For an agent compatible with these basic therapeutic principles, our attention was drawn to the nutritional L-glutamine, which has been shown to contribute favourably to the integrity of the mucosal epithelium of the digestive tract, promote mature collagen growth, and accelerate healing [7,8]. The study was designed to investigate the curative effect of L-glutamine on corrosive burns of the esophagus and to demonstrate that L-glutamine is beneficial in preventing the formation of corrosive esophageal strictures, reducing scar formation and deposition of immature collagen.

MATERIALS and METHODS

Ethics Committee Approval

Ethics committee approval was obtained from the Ege University Animal Experiments Local Ethics Committee (decision number: 2010-157, date: 24.12.2010).

Subjects

Rats were obtained from Ege University Animal Experiments Laboratory. Thirty Spraque-Dawley rats weighing 150 to 250 g were used. The rats were divided into three groups: the unburned esophagus group (control group, n=10), the burned esophagus group (EB, n=10) and the burned esophagus + L-glutamine supplemented group (EB GLN, n=10).

Animal Model of Corrosive Esophagitis

In this study, Gehanno and Guedon [9] esophageal burn model was used. The feeding of all rats was stopped 12 hours before the experiment. Afterward, anesthesia was administered by intraperitoneal injection of 0.75 mg/100 g ketamine and 0.15 mg/100 g xylazine hydrochloride. The stomach was accessed through laparotomy incision under sterile operating conditions and taken out through the incision. The distal part of the esophagus was suspended together with a 1.5-2 cm segment of the abdominal esophagus using 3/0 Vicryl sutures, and a bulldog clamp was used for the esophagogastric junction. An 8 Fr orogastric feeding tube was inserted from the pharynx into the distal lumen of the esophagus 0.5 cm away from the cardia. A 3/0 Vicryl thread was tied around this feeding tube to prevent proximal reflux.

The distal and proximal ends of the esophagus were clamped, and 1 mL of 20% sodyum hidroksit solution was instilled in the EB and EB-GLN groups. In the other group, 1 mL of 0.9% NaCl solution was given. After 1 minute, the solutions were aspirated and the clamps were released. Subsequently, the abdominal incision was closed full-thickness using a 3-0 silk suture. Afterward, the rats were fed with tap water and an aqueous solution of a standard rat chow delivered without irritating the esophagus. All animals were kept in the same areas throughout the study and were fed with rat chow and water ad libitum.

Application of L-glutamine and Termination of the Experiment

The dose of L-glutamine for rats was determined as 1 g/kg/day. L-glutamine was given to the EB-GLN group by gavage for 21 days as a single dose. The rats in the control and EB groups also received 0.9% NaCl solution using the same protocol. The experiment was finished after 22 days. The rats were sacrificed by cervical dislocation. The esophagus of the subjects was removed and placed in 10% formaldehyde.

Histopathological Examination

The samples were examined in paraffin blocks. Sections from paraffin (4-5 μm thick) were stained with hematoxylin and eosin and Gömöri trichrome and examined under a Nikon Optiphot-2 light microscope. Tissue damage was assessed using collagen deposition, ulceration and inflammation scoring, and the stenosis index (SI) [10]. Histopathological evaluation criteria are shown in Table 1. The SI was calculated using the formula SI = wall thickness/lumen diameter. The esophageal lumen was measured using an x4 ocular micrometer lens. Results of esophageal wall thickness and lumen diameters measured from 4 different sites were recorded.

Statistical Analysis

For the analysis of variance between groups, the Kruskal-Wallis test and Mann-Whitney U test with

Bonferonni correction were used. Histopathological scores were analyzed using χ^2 and Fisher's Exact tests. P<0.05 was considered statistically significant. Statistical calculations were performed using SPSS statistical software version 19.

RESULTS

Mortality and Body Weight

Two rats exited on day 6 (EB group) and day 13 (EB-GLN group) were not included in the study. The body weights of all rats were measured at the beginning and end of 3 weeks (Table 2). The control group of unburned rats gained weight. Weight loss in the group with burns that received L-glutamine was lesser compared to those without.

Results of Histopathological Evaluation

In the histopathological examination, there was little inflammation in 1 out of 10 rats in the control group without any collagen deposition, ulceration, or damage to the muscular mucosa in the tunica muscularis (Figure 1). In the EB group, mild inflammation was detected in six, and significant inflammation, and muscular mucosal damage in three rats. Six rats had ulcerations. Damage or collagen deposition in the tunica muscularis was grade 1 in one rat and grade 2 in the other eight rats (Figure 2).

Two rats in the EB-GLN group had inflammation and damaged muscularis mucosa. No ulceration was observed in the EB-GLN group (Figure 3). Collagen depositions in the tunica muscularis were grade 1 in one rat and grade 2 in two rats in the EB-GLN group where all injuries caused by induced CE were statistically significantly alleviated (p<0.05) compared with the EB group (p<0.05).

Statistically significant differences were observed between all groups regarding the SI (p<0.05). The stenosis indices in the control group and EB-GLN group were significantly lower compared to the EB group (p<0.05). In addition, the SI in the control group did not differ significantly compared to the EB-GLN group (p=-0.624). Comparative results of histopathological examinations and SI are shown in Table 3.

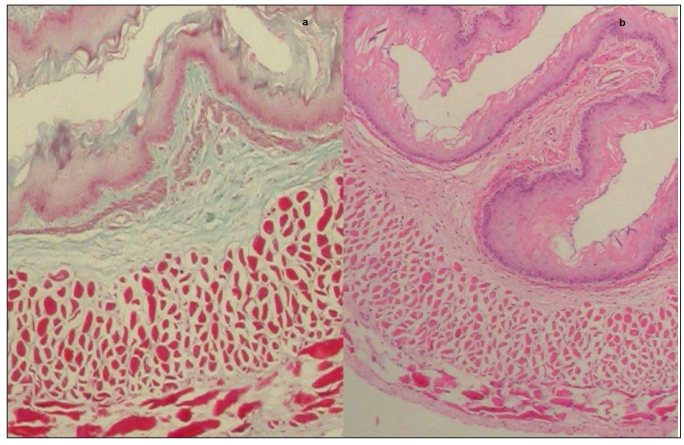

Figure 1. The sequence of the oesophagus of the control group. [minimal inflammation in one magnification, x100; staining, (a) Gomori trichrome and (b) hematoxylin-eosin]

Table 1. Histopathological evaluation criteria

Inflammation		None	0
Little		1	
Mild		2	
Marked		3	
Ulceration		None	0
Present		1	
Increase in collagen deposition	Submucosa	None	0
		Mild (submucosal collagen at least twice the thickness of muscularis mucosa)	1
		Marked (submucosal collagen more than twice the thickness of muscularis mucosa	2
	Muscularis mucosa	No damage	0
		Damage present	1
	Tunica muscularis	No damage or collagen deposition	0
		Damage present	1

Histologic characterization of vaginal vs. abdominal surgical wound healing in a rabbit model.[10]

DISCUSSION

Our experimental study aimed to show whether oral L-glutamine supplementation prevents fibrosis and stricture formation after corrosive esophagitis. The hypothesis of the study is based on the anti-inflammatory and protective effects of L-glutamine on intestinal, myocardial and liver tissues demonstrated in previous studies [7,11]. As a potent agent to help heal tissue damage, several meta-analyses have evaluated the therapeutic efficacy of glutamine in other inflammatory

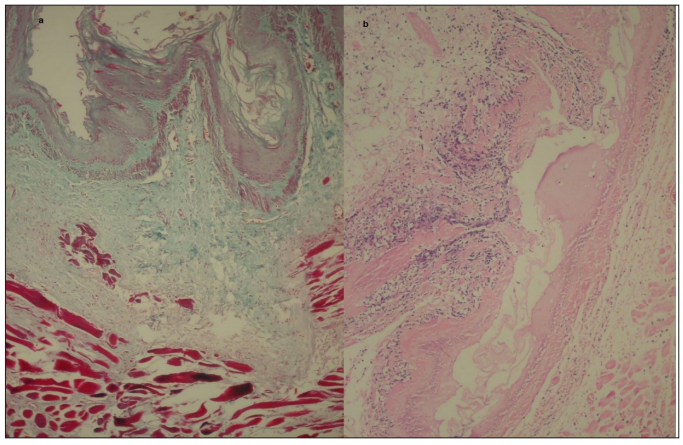

Figure 2. Widespread collagen accumulation was observed in the submucosal and muscular layers and inflammation and ulceration in EB group [magnification, x100; staining, (a) Gomori trichrome and (b) hematoxylin-eosin]

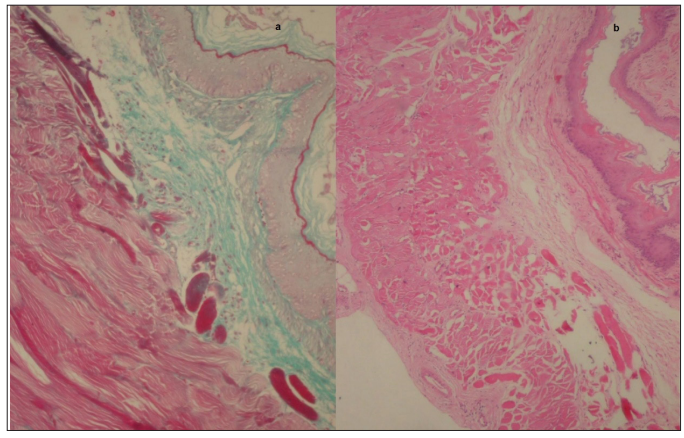

Figure 3. Reepithelization, minimal inflammation and regular mucosal, submucosal and muscular structures in EB-GLN Group. A slight increase in collagen accumulation was observed in the muscularis mucosa [magnification, x100; staining, (a) Gomori trichrome and (b) hematoxylin-eosin]

Table 2. Body weights of the animals in each group

Group	Mean weight at day 1	Mean weight at day 21	Change (%)
Control	172.5 g	173.5 g	0.59% (+)
EB	164.09 g	153.5 g	6.45% (-)
EB-GLN	166.18 g	164.5 g	1.01% (-)

EB: Esophagus burn, EB-GLN: Esophagus burn glutamin administered

Table 3. Comparison of stenosis index and histopathological evaluations

Histopathological findings	Control	EB group	EB-GLN group	P value (EB versus EB-GLN)
Inflammation	1/+ 9/-	9/+ 0/-	2/+ 7/-	0.001
Ulceration	0/+ 10/-	6/+ 3/-	0/+ 9/-	<0.001
Injury of muscularis mucosa	0/+ 10/-	9/+ 0/-	2/+ 7/-	0.015
Collagen deposition in tunica muscularis				
Grade 0	10/+	0/+	6/+	0.005
Grade 1	0/+	1/+	1/+	
Grade 2	0/+	8/+	2/+	
Stenosis index	0.25 (+\-0.05)	0.72 (+\-0.07)	0.36 (+\-0.06)	0.013

EB: Esophagus burn, EB-GLN: Esophagus burn glutamin administered

diseases such as Crohn's disease, oral mucositis, and respiratory disease [12,13].

Studies have shown that under stress, serum glutamine level generally decreases and the integrity of the intestinal tract epithelium is impaired [14]. In rats with intestinal ischemia, treatment with glutamine has been shown to suppress lactate dehydrogenase levels and reduce the incidence of bacterial translocation [15]. In rats exposed to hypobaric hypoxia, glutamine treatment has become effective in alleviating intestinal damage by reducing inflammatory cytokines (TNF-α and IL-6) [16]. Accordingly, the antioxidant and anti-inflammatory effect of L-glutamine protects cells against lipid peroxidation, which is the beginning of many pathological stages [17].

Corrosive strictures with alkaline agents occur in three phases. In the first phase - the acute necrotic phase which onsets 1-4 days after an injury - an intense inflammatory reaction in the local tissue and coagulation of intracellular proteins cause cell necrosis. In the second phase (3-12 days following injury); ulceration and granulation tissue fill the defective area. In the final phase of the wound healing process (3rd week of injury); the connective tissue contracts and narrows the esophagus [18]. Fluid therapy, antireflux therapy, antibiotics, and steroid therapy are being currently used to reduce chemical trauma and bacterial infection in the acute phase, prevent fibroblastic activity, and collagen deposition in the long term [5,19].

In our study, we observed that dietary L-glutamine supplementation in the caustic burn group was able to prevent chemical damage by decreasing the deep penetration of chemical damage thro h increasing epithelialization in the esophageal lumen in the acute phase of corrosive damage. Our data have shown that, compared to the EB group, the EB-GLN group had a significantly lower SI, lesser inflammation, and collagen deposition in the submucosa. Histopathological findings correlate with the results. Lesser weight loss was detected in the group that received nutritional support with glutamine compared to the other groups. It has been shown that oral glutamine use may have an important role in reducing weight loss and the need for analgesics in aerodigestive system malignancies with acute radiation toxicities [20].

Study Limitations

One of the limitations of our study is that although the burn model was created by taking a previously defined model as an example, esophageal stricture formation may not always be observed after this injury. The limited number of subjects used also reduces the probability of esophageal stricture occurring. If contrast imaging could be done, it might create data to support the predictive value of the SI.

CONCLUSION

In conclusion, our results have shown the efficacy of L-glutamine therapy as an adjunct to conventional therapy in the model of corrosive esophageal burn. L-glutamine has been shown to significantly reduce the degree of fibrosis and improve histopathological damage. Our study's the first experimental research that used L-glutamine in the treatment of corrosive esophageal burns. More detailed animal and/or clinical studies are needed to support these results.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Ege University Animal Experiments Local Ethics Committee (decision number: 2010-157, date: 24.12.2010).

Informed Consent: Informed consent is not required.

Peer-review: Externally peer-reviewed.

Author Contributions

Surgical and Medical Practices: Ö.O., G.D., O.A.A., Concept: Ö.O., O.A.A., A.O., Design: Ö.O., O.A.A., A.O., M.H., Data Collection and/or Processing: Ö.O., H.E., M.H., Analysis and/or Interpretation: Ö.O., G.D., Literature Search: Ö.O., O.A.A., M.C., H.E., M.H., Writing: Ö.O., O.A.A., M.C., H.E., A.O.

Conflict of Interest: The authors have no conflict of interest to declare.

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