THERAPEUTIC EFFECT OF L-ARGININ ON INDUCED CORROSIVE ESOPHAGEAL BURNS IN THE RATS

Yavuz Y.*, Sözüer E., Yürümez Y.*, Avsaroğulları L.***, İkizceli İ.***, Canöz Ö.****,**

** Afyon Kocatepe University of Medical School, Department of Emergency Medicine*
*** Erciyes University of Medical School, Department of General Surgery*
**** Erciyes University of Medical School, Department of Emergency Medicine*
***** Erciyes University of Medical School, Department of Patology*

Yücel Yavuz : Afyon Kocatepe University of Medical School, Departent of Emergency Medicine 03100 AfyonKarahisar
Phone: +90-272 241 20 65 Fax: +90-272 214 20 60 E-mail : yyavuzmd@yahoo.com

This experimental study was supported by the Research Foundation of Erciyes University and approved by Medicine Faculty Academic Board with permission number of 01-11-84, was conducted at Hakan Çetinsaya Experimental Research Center and at the Pathology Laboratory.

KOROZİV ÖZOFAGUS YANIĞI OLUŞTURULAN RATLARDA L-ARGİNİN' İN TEDAVİ EDİCİ ETKİSİ

ÖZET

Koroziv özofagus yanıklarında akut dönemin atlatılmasından sonra hasta ve hekimi ilgilendiren en önemli sorun striktür gelişmesidir. Koroziv özofagus yanıklarının tedavisinde esas hedef striktür oluşmasının engellenmesidir. Bizde çalışmamızda koroziv özofagus yanığı oluşturulan ratlarda L-argininin iyileştirici etkisini araştırdık.

Çalışmamızda toplam 60 adet rat kullanıldı ve her biri onar rattan altı gruba ayrıldı. İlk üç grupta deney 48. saate sonlandırıldı. Son üç grupta ise 28. günde sonlandırıldı. Grup I ve IV'deki ratlarda yanık oluşturulmadı ve tedavi edilmedi. Grup II ve V'deki ratlarda % 10 NaOH ile yanık oluşturuldu fakat tedavi yapılmadı. Grup III ve VI'daki ratlarda % 10 NaOH ile yanık oluşturuldu ve ilki yanık oluşturulduktan yarım saat sonra olmak üzere 250 mg/kg/gün L-arginin günde tek doz verildi. Grup III'de tedavi iki kez yapıldı. Grup VI'de ise tedavi bir hafta yapıldı. İlk üç grupta 48.saatte son üç grupta ise 28. günde özofagus distal 1,5 cm'lik kısım alınarak histopatolojik olarak incelendi. L-arjinin ile tedavi edilen grupta ikinci günün sonunda ödemin ve enflamasyonun belirgin olarak azaldığı mikroskobik olarak tespit edildi. L-arjinin ile tedavi edilen grupta 28. günün sonunda ortaya çıkan submukozal kollajen artışı'nın belirgin olarak azaldığı mikroskobik olarak tespit edildi. Sonuç olarak, L-argininin koroziv özofagus yanıkları üzerinde iyileştirici etkisi olduğunu düşünmekteyiz.

ANAHTAR SÖZCÜKLER : Koroziv yanık, Özofagial striktür, L-Arginin, Tedavi

SUMMARY

In corrosive esophageal burns the most important issue after the acute stage for both patient and doctor is the prevention of stricture formation. In this study, we tested the therapeutic effect of L-arginine on the occurrence of corrosive esophageal burns in a rat model.

Sixty rats were diveided into six groups with ten rats in each group. The first three groups were observed for 48 hours, and the last three groups for 28 days. The rats in Groups I and IV were exposed to neither the corrosive substance nor treatment. The corrosive esophageal burns were induced in the rats in Groups II, III, V and VI by using %10 NaOH solution. Groups II and V were not treated, whereas Groups III and VI were given 250mg/kg of L-arginine once a day, the first dose being administered 30 minutes after the corrosive burn was induced. Treatment in Group III was administered twice, whereas treament in Group VI treatment was given every day for a week. At the end of the study, after the animals were sacrificed, all animals had the distal 1.5 cm of esophagus removed and histopathologically studied. The oedema and inflammation in the L-arginine groups were healed, as visualized under the microscope, by the end of 48 hours. At 28 days, the L-arginine group had significantly less collagen increase in the submucosa. L-arginine has a beneficial therapeutic effect on corrosive esophageal burns.

KEY WORDS : Corrosive burn, Esophageal stricture, L-arginine

INTRODUCTION

Corrosive burns, caused by caustic agents, of the upper gastrointestinal system (GIS) is an important problem and appears most frequently between two and three years old children [1,2].

One of the serious complication with the ingestion of corrosive agents is esophageal stricture formation[2,3]. The main aim in the treatment of corrosive esophageal burns is to prevent the stricture formation[4]. There are various treatment methods to prevent stricture formation after burns, but their results are still under discussion and new treatment methods are under investigation[2,5,6,7]. L-arginin accelerates the treatment of wounds, plays role in Gastrointestinal (GI) cells metabolism and decrease or even reconstruct the hazardous effect of Nitric Oxide Sentaz (NOS) inhibitor on GIS mucosa completeness and on blood flow[8,10]. This study aims on preventing the formation of stricture in corrosive esophageal burns by using the therapeutic effect of L-arginin in rats.

MATERALS AND METHODS

This experimental study was supported by the Research Foundation of Erciyes University and approved by Medicine Faculty Academic Board with permission number of 01-11-84, was conducted at Hakan Cetinsaya Experimental Research Center and at the Pathology Laboratory.

Experimental groups : Six randomized groups , each 10 rats, were studied.

Group I (Sham group, 48 hours): In this group of rats, neither corrosive esophageal burn was induced nor L-arginin treatment was given. In this group, at the end of 48 hours 1.5 cm of esophageal distal was removed and histopathologically studied.

Group II (Control group, 48 hours): In this group of rats, esophageal corrosive burn was induced by using NaOH solution. But, L-arginin was not given. In this group, at the end of 48 hours 1.5 cm of esophageal distal was removed and histopathologically studied.

Group III (Experimental group, 48 hours): In this group of rats, esophageal corrosive burn was induced by using NaOH solution and treated with L-arginin. In this group, at the end of 48 hours 1.5 cm of esophageal distal was removed and histopathologically studied.

Group IV (Sham group, 28 days): In this group of rats, neither corrosive esophageal burn was induced nor L-arginin treatment was given. In this group, at the end of 28 days 1.5 cm of esophageal distal was removed and histopathologically studied.

Group V (Controll group, 28 days): In this group of rats, esophageal corrosive burn was induced by using NaOH solution. But, L-arginin was not given. In this group, at the end of 28 days 1.5 cm of esophageal distal was removed and histopathologically studied.

Group VI (Experimental group, 28 days): In this group of rats, esophageal corrosive burn was induced by using NaOH solution and treated with L-arginin. In this group, at the end of 28 days 1.5 cm of esophageal distal was removed and histopathologically studied.

Experimental Design

After 12 hours of fasting before surgery, the rats in all groups were anesthetised by ketamin hydrochloride with 50 mg/kg (Ketalar® 50mg/ml 10ml flakon- Eczacıbaşı). The 4 ml of normal saline was slowly given to stomach through a orogastric tube. Then, the rats in groups I and IV received 0.5 ml of normal saline through the orogastric tube via infusion pump (LifeCare Pump®-Abbott) for two minutes. The rats in groups II, III, V and VI received 0.5 ml 10% of NaOH through the orogastric tube via infusion pump for two minutes. After the procedere, the esophagous of rats in all groups rinsed with 0.5 ml of normal saline through orogastric tube. After 30 minutes of completing the experiment, the groups I and II received 0.5 ml of normal saline whereas the group III received 250mg/kg L-Arginin (SIGMA®) diluted with 0.5 ml of normal saline via the orogastric tube. The same procedure was repeated after 24 hours. 48 hours later from the beginning of the experiment, the rats in groups I, II and III were sacrificed with high dosage of ketamin. Then, 1.5 cm of esophageal distal was removed for histopathological studies. The rats in Group IV and V received 0.5 ml SF trough orogastric tube after 30 minutes of the first experiment and in the following days at the same time for seven days to esophagous. The rats in Group VI received (250 mg/kg) L-Arginin trough orogastric tube after 30 minutes of the first experiment and in the following days at the same time for seven days to esophagous. The rats at the end of 28th day were sacrificed under high dose of ketamin and then 1.5 cm of esophageal distal was removed for histopathological studies.

Histopathological Analysis

The removed 1.5 cm of esophageal distal was fixed by 10% formalin. After routin tissue procedures all tissues were embedded in parafin where 5-8 micron thick parafin sections were prepared. Coloring procedure was carried out by hemotoksilen-eosin and masontokrum. Colored preperats were evaluated and scored under microscope according to Table 1[11,12]. In histopathologic evaluation

we looked for oedema of submucosa, inflammation of submucosa, increase in submucosal collagen (ISC), damage in muscularis mucosa (DMM), and damage and collagen deposition in tunica muscularis (DCDTM).

Statistical Analyses

Analyses were performed on a desktop computer using statistical analysis software (SPSS release 10.0). Statistical analysis of the histopathologic scores for groups I, II and III in between and for groups IV, V and VI in between was performed with Kruskal-Wallis test. Finally, groups were compared with each other with Mann-Whitney U test.

RESULTS

The results we have obtained are as following:

Oedema

The groups (Groups I, II and III) compared at the end of second day statistically differed from each other ($p<0.01$). This difference was especially very significant between Groups I and II ($p<0.01$) and between Groups II and III ($p<0.05$) wehereas the difference between the Groups I and III was not significant ($p>0.05$, Table 2) (Figure 1).

Inflammation

The groups (Groups I, II and III) compared at the end of second day statistically differed from each other ($p<0.01$). This difference was especially very significant between Groups I and II ($p<0.01$) and between Groups II and III ($p<0.05$) wehereas the difference between the Groups I and III was not significant ($p>0.05$, Table 2) (Figure 1).

Damage to the muscularis mucosa

The groups (Groups IV, V and VI) compared at the end of 28th day statistically differed from each other ($p<0.01$). However it is worth to note that only in one subject in Group V damage in muscularis mucosa was not observed. This difference was especially very significant between Groups IV ile V ($p<0.01$) wehereas the difference between Groups IV ile VI and between the Groups I and III was not significant ($p>0.05$, Table 3) (Figure 1).

Increase in submucosal collagen

The groups (Groups IV, V and VI) compared at the end of 28th day statistically differed from each other ($p<0.01$). This difference was especially very significant between Groups IV and V ($p<0.01$) and between Groups V and VI ($p<0.05$) wehereas the difference between the Groups IV and VI was not significant ($p>0.05$, Table 3) (Figure 1).

Damage and collagen deposition in the tunica muscularis

The groups (Groups IV, V and VI) compared at the end of 28th day statistically differed from each other ($p<0.01$). This difference was especially very significant between Groups IV ile V ($p<0.01$) wehereas the difference between Groups IV ile VI and between the Groups I and III was not significant ($p>0.05$, Table 3) (Figure 1).

DISCUSSION

Corrosive esophageal burns in acute and chronic period can cause many complications even deaths[4,12]. Both specific and general operation is needed for early and intensive complications such as esophageal perforation, mediastinit, gastritis, gastric perforation, laryngeal oedema and pulmonary oedema[12]. The stricture development is the main problem related to patient and physician after the kicking of an acute period[13,14]. Necessary treatment should be done to prevent this late period complication. Stricture is observed from patients who have the second or third degree esophageal burn[4].

Almost all of the contemporary treatment methods recently applied is based on the information obtained from animal experiments[15]. According to the experimental observations on rats, it was observed that, although 3,8% NaOH concentration causes necrosis of the mucosa, submucosa and rarely muscle leaves in 10 seconds[5,16,17], 26,6% NaOH can totally destroy esophagus wall in 10 seconds. 10,7% NaOH can cause necroses in mucosa, submucosa and muscularis stratum[5,16,17]. Therefore we used 10% NaOH in our observation.

Animal experiments have shown that in the following seconds of the touch of corrosive substance to tissue, erratum and oedema occur. Tissue oedema and hyperemia occur straightforward and can continue about 48 hours. Acute inflammatory period can keep on 1-4 days. However, inflammatory response is the most intensive in 24-48 hours. We also thought that the second day inflammation and oedema would be more intensive, and hence we stopped the experiment at the end of the second day. We obviously determined oedema and inflammation in the experimental group. We also established expressive decrease in oedema and inflammation in the treatment group. In the study of Berthet et al[18] evaluation of oedema and inflammation was done on the second day and modarete oedema and inflammation was observed.

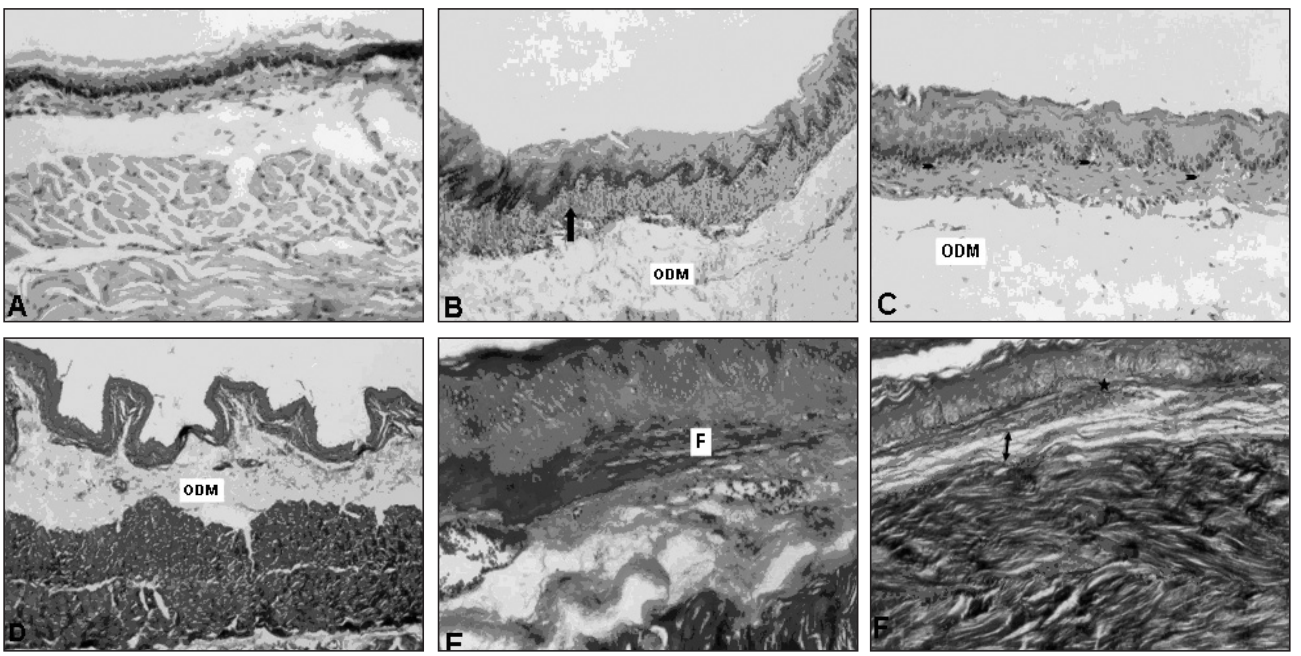
In the experimental observations skatrization phase continues between the second and third weeks and totally completes in the fourth week[1,19]. Therefore, as it is in the other studies, we also did microscopic inspection and found the increase in submucosal collagen and, damage and collagen deposition in the tunica muscularis at the end of the fourth week[11,12].

In a study carried out by Brzozowski et al[20], acute gastritis mucousal damage was occurred and it was determined

that intragastric L-arginin had mucous protective effect. Stomach blood flow was also measured in the same work and it increased significantly by the effect of L-arginin. On the other hand, Avşaroğulları et al[21] determined that L-arginin shows an obvious mucousal protective effect.

On the submucousal and muscular layers succesive enflamatuar reactions may develop and ulcers may form depending on the corrosive esophagus burns. There exist transmural thrombosis in microscopic inspection and this produces early cell deaths in necrotic phase[1,22]. Koloğlu et al[23] studied the effect of heparin on stricture development in corrosive esophagus burn in an experiment done on rats. Thrombosis of submucousal veins in alkali burns increases mucousal ischemia and forms necrosis in wider zone. Observation was done with the concern of heparin can give benefits by prevention of thrombosis developing. As a result it was observed that heparin histopathologicaly decreases the level of ICS and hydroxyproline.

L-arginin shows its effect by transformation to NO via NO biosynthesis. L-arginin accelerate wound healing, takes a role in GI cells metabolism, and decreases or turns over the harmful effects of NOS inhibitors on the integrity of GIS mucosa and blood flow[9,10,11]. It is known that NO has an important role of microcirculation regulation in both physiologic and pathologic circumstances[24]. As a result, in this study we obtained statistically significant results that L-arginin decreases oedema at the 48th hour and ICS on the 28th day. We established that L-arginin decreases collagen developing in corrosive esophagus burns and hence it prevents stricture development. Since our results are supported by other observations, we thought that it can clinically be used.



A- Normal histological appearances in osefagus of control rat (H&E, X20).

B- Group II rat, ODM; oedema, inflammation,Group rat, (H&E, X40).

C- Group II rat, ODM; oedema, Group rat, (H&E, X100).

D—Group III rat, oedema (Tricrom, X40).

E- Group V, Increase in submucosal collagen, F: Fibrosis Group rat, (Tricrom, X200).

F- Group VI rat, Damage to the muscularis mucosa and Increase in submucosal collagen (Tricrom, X200).

➨ İnflammation

* Damage to the muscularis mucosa

↔ Increase in submucosal collagen

Figure 1:

Criterion	Score
Oedema and inflamation	
Mild	0
Moderate	1+
Severe	2+
Increase in submucosal collagen	
None	0
Mild (submucosal collagen at least twice the thickness of the muscularis mucosa)	1+
Marked (submucosal collagen more than twice the thickness of the muscularis mucosa)	2+
Damage to the muscularis mucosa	
None	0
Present	1+
Damage and collagen deposition in the tunica muscularis	
None	0
Mild (collagen deposition around the smooth muscle fibers)	1+
Marked (same as mild, with collagen deposition replacing some of the fibers)	2 +

Tablo I. Criteria for histopathologic Evaluation

	Group I Median (min-max)	Group II Median (min-max)	Group III Median (min-max)	chi-Square	P
Oedema	0 (0 - 0)b	1.0 (0 – 2.0)ac	0.5 (0 – 1.0)b	16.41	< 0.01
Inflammation	0 (0 - 0)b	1.0 (0 – 1.0)a	0.5 (0 – 1.0)	10.47	< 0.01
DMM	0 (0 - 0)	0 (0 – 1.0)	0 (0 – 0)	3.24	> 0.05
ISC	0 (0 - 0)	0 (0 – 1.0)	0 (0 – 0)	2.14	> 0.05
DCDTM	0 (0 - 0)	0 (0 – 0)	0 (0 – 0)	.00	> 0.05

*p<0,05 concerned as expressive.
a: Shows difference to Group I. b: Shows difference to Group II. c: Shows difference to Group III.

Table II : Statistical results of the groups at the end of the second day

	Group IV Median (min-max)	Group V Median (min-max)	Group VI Median (min-max)	chi-Square	P
Oedema	0 (0 - 0)	0 (0 – 0)	0 (0 – 1.0)	2.14	> 0.05
Inflammation	0 (0 - 0)	0 (0 – 0)	0 (0 – 0)	2.00	> 0.05
DMM	0 (0 - 0)b	1.0 (0 – 1.0)a	0 (0 – 1.0)	16.00	< 0.01
ISC	0 (0 - 0)bc	1.0(1.0–2.0)ac	1.0 (0 – 1.0)ab	20.84	< 0.01
DCDTM	0 (0 - 0)b	1.0 (0 – 2.0)a	0.5 (0 –1.0)	12.97	< 0.01

*p<0,05 concerned as expressive.
a: Shows difference to Group I. b: Shows difference to Group II. c: Shows difference to Group III.

Table III : Statistical results of the groups at the end of the 28th day

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