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Protective effect of early placement of nasogastric tube with solid dilator on tissue damage and stricture formation after caustic esophageal burns in rabbits☆,☆☆

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ABSTRACT

Background: The ingestion of caustic substances remains an important public health issue worldwide. Children represent 80% of the ingestion injury population globally. Accidental alkaline material accounts for most caustic ingestions. There is no conclusive evidence of tissue damage and stricture protection of a nasogastric-tube with a solid dilator in the literature, therefore it was hypothesized that early intraesophageal tube placement does not cause additional histopathologic damage and prevents strictures.

Methods: An exploratory study on experimental caustic esophageal burns in a rabbit model was designed. In the treated group a silicone tube was placed immediately after causing the burns, while the untreated group followed the natural course of the burn. On the twenty-second day, an esophagectomy was performed on all animals for microscopic (Histopathologic Damage Score and Stenosis Index) and macroscopic analysis.

Results: Forty animals were randomly divided into two groups. The Histopathologic Damage Score was 3.7 ± 1.1 in the treated group versus 3.9 ± 1.2 in the untreated group ($p = .9690$). The Stenosis Index was 0.6 ± 0.1 in treated rabbits versus 2.3 ± 0.2 in untreated ($p < .0001$).

Conclusion: The early placement of an intraesophageal tube with solid dilator prevents stenosis formation and does not produce greater tissue damage.

The ingestion of caustic substances remains an important public health issue worldwide despite educational and regulatory efforts intended to reduce its occurrence [1]. These injuries are still increasing in developing countries [2], related to social, economic, and educational factors and mainly to a lack of prevention [3,4]. Worldwide, children represent 80% of the ingestion injury population [5], primarily because of accidental ingestion [6].

Alkaline material accounts for most caustic ingestions in Western countries [7]. Alkalis combine with tissue proteins, cause liquefactive necrosis and saponification, and penetrate deeper into tissues. Additionally, alkali absorption leads to thrombosis in blood vessels, impeding blood flow to already damaged tissue [8,9].

Experimental findings suggest that arteriolar and venular thrombosis with consequent ischemia may be more important than inflammation in the pathogenesis of acute corrosive injury. Four to seven days after ingestion, mucosal sloughing and bacterial invasion are the main findings. Simultaneously, granulation tissue appears, and ulcers become covered by fibrin. Perforation may occur during this period if ulceration exceeds the muscle plane. Fibroblasts appear at the injury site around day four, and around day five, an "esophageal mold" is formed, consisting of dead cells and secretions. Esophageal repair usually begins on the tenth day; stenosis formation occurs on day twenty-one and is complete from one to two mounts after ingestion [10]. The tensile strength of the healing tissue is low during the first three weeks since collagen deposition may not begin until the second week. Scar retraction begins by the third week and may continue for several months, resulting in stricture formation and shortening of the involved segment of the gastrointestinal tract. Additionally, lower esophageal sphincter pressure becomes impaired, leading to increased gastroesophageal reflux (GER), which in turn accelerates stricture formation [11]. When it comes to deciphering the systemic and cellular mechanisms involved in the hypermetabolic response to burn injury, evidence supports the use of larger animals. To resolve the complexities and high costs associated with large animal burn models, rabbits are not only relatively large animals that maintain a metabolic relevance to humans, they are an appropriate animal model for studying burns induced by hypermetabolism and their pathological alterations in energy homeostasis because they share with humans several aspects of their metabolism, such as similarities in composition of Apo lipoprotein B (Apo B)-containing

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lipoproteins, hepatic production of Apo B 100-containing very low dense lipoproteins (VLDL), human-like Apo B, and low hepatic lipase activity. It has also been shown that rabbits present elevated REE (resting energy expenditure) levels post-thermal injury, which is a characteristic metabolic feature in burn patients. [12]. Although the biomechanical and histological properties of two-layer esophagi animals (particularly rodents) are still insufficient, the similarity in biological reaction time after burns between rabbit and human esophagi is well documented [13].

Immediate treatment is usually conservative, as the definitive extent of the injury is determined within minutes after ingestion, which is why a preventive stricture strategy is critical. Though a tube may be helpful to ensure patency of the esophageal lumen, the tube itself could contribute to the development of long strictures or esophageal perforation and routine use is not uniformly recommended [1,14]. Any esophageal catheterization may be a nest for infection, and nasogastric placement may worsen gastroesophageal reflux in this patient population, with a consequent delay in mucosal healing. Should a tight stricture develop, positioning a tube has the advantage of providing a lumen for dilatation. Therefore placing a tube may be considered after caustic injuries, but the decision should be made on a case-by-case basis and it is essential to proceed with caution.

There are no conclusive studies in the literature on the effects of an intraesophageal tube with a solid dilator placed immediately after burns either on tissue damage, or stricture prevention. Therefore, the hypothesis that immediately placing an intraesophageal tube would not cause additional histopathologic damage and might even prevent strictures was proposed.

The aim of this work was to describe in an experimental caustic burn model the effect of early intraesophageal tube placement on Histopathologic Damage Score and Stenosis Index.

1. Materials and methods

1.1. Ethics statement

The study was approved by the Animal Ethics Institutional Committee, Faculty of Medical Sciences, National University of Cordoba (Argentina) and conformed to the Guide for the Care and Use of Laboratory Animals (U.S. NIH Publication # 85-23).

1.2. Study groups

An exploratory experimental study on a rabbit model was designed.

Forty prepubertal male California four to five-month-old rabbits weighing between 2400 and 2650 g were randomly allocated into two groups. The normal dimension of a four- to five-month-old rabbit's esophagus is 13–15 cm in length and 10–12 mm in diameter (2–3 mm in wall thickness and 4–6 mm in lumen diameter).

All rabbits were kept in conditions of 22 °C ± 2 °C, with a controlled light cycle (twelve-hour day, twelve-hour night). The animals were fed with standard balanced food pellets and water *ad libitum*.

In the control group the esophagus was injured and left untreated. In the treated group, the esophagus was injured and therefore received immediate treatment by means of introducing of a malleable silicon nasogastric tube with a metal mandrel through the nose, which had a radiopaque silicon solid dilator mounted collinearly in its middle part, intended to be located in the injured area, fixed firmly by the elasticity and surface tension of the materials, inside the nasogastric tube. Once the nasogastric tube was placed, the guide was removed, and surgically affixed. This device was designed and manufactured by Silmag Biomedical Products Co. (Cordoba, Argentina) (Fig. 1). The distal tail of a nasogastric tube lodged in the stomach and the proximal tail was fixed at the nostrils (please see the schema in Fig. 2). The solid silicone dilator was 9 cm in length and 2 French (0.66 mm) in diameter.

The animals were fasted twelve hours before the procedure. Each rabbit was anesthetized using acepromazine, ketamine, xylazine and atropine intravenously. Animals received sufficient fluids during the procedure (0.9% sodium chloride solution and 5% dextrose). The vital signs were monitored until complete anesthetic recuperation. Subsequently the rabbits received Ibuprofen 5 mg/kg IV every 6–8 hours (Actron™—Bayer Labratories) according to necessity [15], and Cephalexin 25 mg/kg IV every 6 hours (Keforal™—Ivax Laboratories) was administrated for fourteen days. The drugs were administered to both groups simultaneously, to avoid differences attributable to their pharmacological effects.

1.3. Experimental esophageal burns model

The following method was used to create experimental esophageal burn: the animals underwent a rigid esophagoscopy and circumferential swabbing on the upper and middle third of the esophagus with 10% NaOH (pH 13.8) for one minute, and then washed with (0.9% sodium chloride solution and 5% dextrose). Immediately the burn was corroborated by esophagoscopy and the tube with permanent solid dilator was placed at the site of the injury in the treated group. Finally a gastrostomy was performed for feeding purposes (Stamm technique) with a silicone button (1.2 cm in length and 18 French in diameter) manufactured by Silmag Biomedical Products Co. (Cordoba, Argentina)

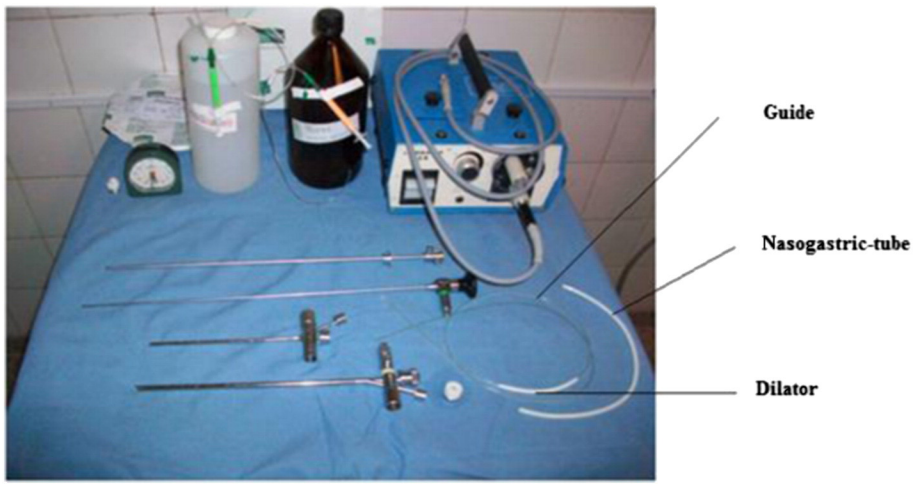

Fig. 1. Silicon nasogastric tube with solid dilator used in the study. Malleable silicon stent with a metal guide and a radiopaque solid silicon dilator mounted in its middle part to be placed at the site of the injury (lower right corner). Silmag Biomedical Products Co™.

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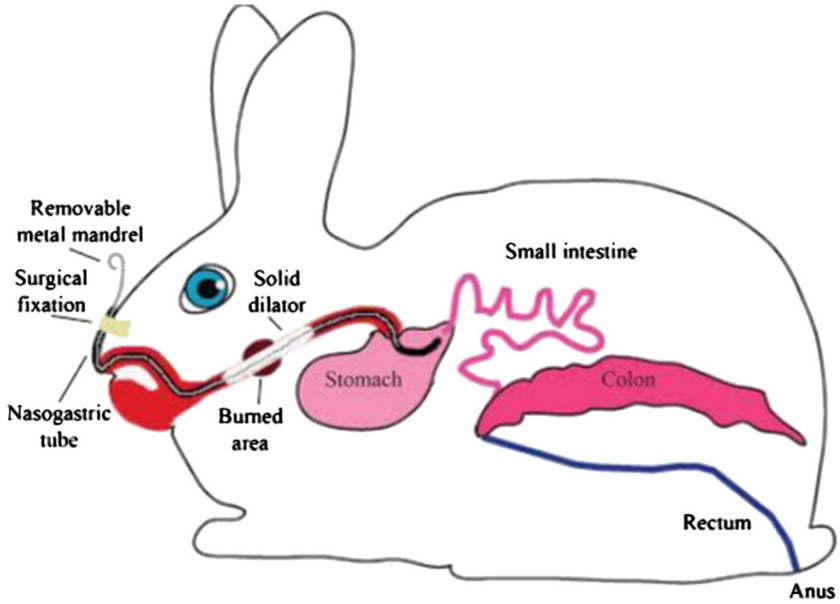

Fig. 2. Schematic representation of the insertion of the nasogastric tube with solid dilator in the rabbit model.

performed only in the untreated group for feeding purposes. Afterward, the rabbits were fed with standard liquid formulation.

1.4. Preparation of animals for histological analysis and Stenosis Index

Twenty-four hours before the end of the experiment all nasogastric tubes were removed. Later, all rabbits were sacrificed after receiving a high-dose of pentothal, and a total esophagectomy was performed. Tissue samples for histopathological analysis were harvested from all animals and fixed in 10% formaldehyde.

1.4.1. Histopathologic evaluation

Histopathologic analysis was performed on day twenty-two; one day later, stenosis formation occurs after experimental lesion [10], by two pathologists in a blind manner, with a previously tested interobserver agreement greater than 80%. Paraffin sections were stained with hematoxylin–eosin and Mason trichrome and examined using a Zeiss Axio Lab A1 (Zeiss Axio Cam MRc5 attachment) light microscope. Tissue damage was scored by Histopathogical Damage Score. The score has three categories: collagen deposition in the submucosa, damage of muscularis mucosa, and collagen deposition in tunica muscularis. The score reflects that damage occurs mainly because the trauma scarring takes place by proliferation of fibroblasts and collagen deposition, with a total of zero to five (see Table 1) [16].

Table 1
Histophatologic Damage Score.

Criterion	Score
Increase in submucosal collagen	
None	0
Mild	1
Marked	2
Damage of muscularis mucosa	
None	0
Present	1
Damage and collagen deposition in the tunica muscularis	
None	0
Mild	1
Marked	2

Modified from Guven et al. [16].

1.4.2. Stenosis Index

The esophageal wall thickness and lumen diameter were measured using a millimetric optic microscope to calculate Stenosis Index, using the formula: Stenosis Index = [wall thickness (mm)]/[lumen diameter (mm)] [17].

1.5. Statistical analysis

The sample size was calculated using Julious' formula [18].

The statistical analysis was made using SPSS statistical software (IBM SPSS version 21). Discrete data were described in percentages and continuous data were described in means ± standard deviation. Differences between the groups were analyzed by χ^2 Fisher's exact test and Mann–Whitney U test. Significance was accepted for $p < .05$.

2. Results

2.1. Mortality

During a period of twenty-one days, thirty-seven rabbits survived (37/40 [92.5%]. One (1/20 [5%]) rabbit in the treated group died, whereas two (2/20 [10%]) rabbits in the untreated group died. No statistical significance existed regarding mortality rates between the groups ($p = 1.000$).

2.2. Weight and feeding

The initial mean weight of the rabbits in the treated group was 2540 ± 238 g versus 2642 ± 169 g in the untreated group, without statistical significance ($p = .2290$). There were significant differences at day twenty-two between treated and untreated rabbits (2682 ± 161 g versus 2349 ± 165 g ($p = .0050$). The treated group was able to feed using the nasogastric-tube from the beginning.

2.3. Macroscopy

During the thoracotomy performed on day twenty two – at the end of experiment – the esophagi of the rabbits in the treated group were macroscopically normal with the exception of minor adhesions. In contrast, those from the untreated group showed more severe adhesions and strictures. The animals did not develop esophageal perforations.

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2.4. Microscopy

Under microscopic examination, no considerable differences were observed between the groups (see Fig. 3).

No statistical significance existed between the groups based on the Histopathologic Damage Score. The Stenosis Index in the untreated group was significantly higher than in the treated group. No differences were evidenced in wall thickness, whereas significant differences were observed in luminal diameter comparing treated and untreated groups. These results are shown in Table 2.

Table 2
Comparison between groups in Histopathologic Damage Score and Stenosis Index, wall thickness and lumen diameter.

Variable	Treated group (x ± sd)	Untreated group (x ± sd)	*P*
HDS	3.7 ± 1.1	3.9 ± 1.1	.9690
SI	0.5 ± 0.1	2.2 ± 0.2	<.0001
WT	2.9 ± 0.8	3 ± 0.8	.4329
LD	6.0 ± 0.2	1.4 ± 0.1	.0004

HDS: Histopathologic Damage Score, SI: Stenosis Index, WT: wall thickness, and LD: lumen diameter.

3. Discussion

In this experimental rabbit caustic esophageal burn model, intraesophageal tubes were placed early in order to prevent histopathologic damage and strictures, a procedure that has not been used before in such tissue damage model. This study demonstrates that early placement of an intraesophageal nasogastric-tube with solid dilator does not cause more tissue damage than controls, and prevents strictures.

Twenty-two days post-burn, sections from treated rabbits seemed healthier than those obtained from untreated rabbits, which also confirmed the tube's protective effect.

Esophageal tissue damage and strictures caused by ingestion of caustic materials are a main clinical concern with an elevated degree of morbidity and mortality. Among clinicians there is considerable controversy about the usefulness of early tube placing to prevent strictures for fear of increasing tissue damage and causing perforations. In general this approach is based on evidence derived from studies where the introduction of the tube occurs considerably before the injury, which would increase tissue friability and consequently risk perforation [19–21].

Several lines of evidence indicate that the regenerative capacity of tissue immediately after a burn depends on the number of surviving uninjured cells capable of initiating the mechanisms needed for tissue restoration [22], therefore the number of surviving uninjured cells is very important to demonstrate that the tube does not increase the initial tissue damage.

It was found that the group treated immediately after the burn with tube placed by esophagoscopy, suffered no more tissue damage than the control group. This fact can be explained in part by the carefully executed esophagoscopic procedure performed in order to place a very soft, thin, delicate silicon tube, causing practically no injury whatsoever when measured using the Histopathologic Damage Score.

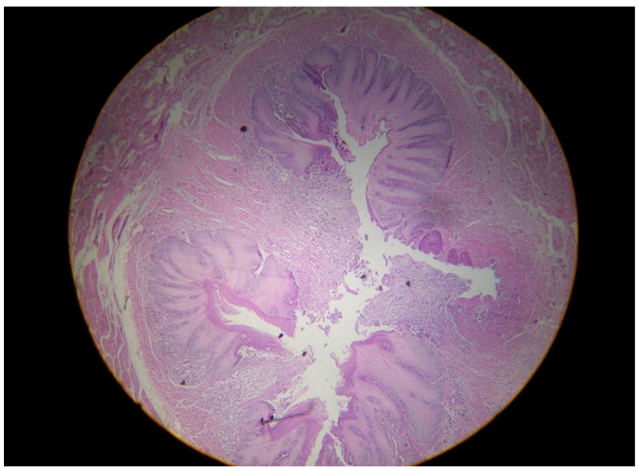

Fig. 3. Representative sample of a microscopic esophageal section. Esophageal section in both groups (treated and untreated) shows similar findings (hypertrophic mucosa, increase in submucosal collagen content, damage to muscularis mucosa and increase in collagen content of tunica muscularis).

The starting time of tube placement in the related studies is generally before the injury [21]. In contrast with these works, in this study the tubes were placed immediately after the burn were created, and before the acute phase occurred, possibly avoiding the initial consequences of inflammatory response, an important mechanism in the pathogenesis of acute corrosive injury, which increased tissue friability and risk of perforation [23]. Given that the metabolic rate of young rabbits is almost 15-fold that of humans, starting the procedure a few minutes after the injury seems equivalent to a few hours in children. Other time-course studies are required to determine the optimal starting time to place the tube after injury.

Experimental research has focused on the healing of caustic esophageal burns without adherences and stricture formation. In this study only minor adhesions were found in the treated group when compared to the control group, which had major adherences. Possibly the effect in the treated group could be explained by a direct inhibition of fibroblastic proliferation in the tubed areas [24]. Certainly ibuprofen may contribute to treatment for its anti-inflammatory effect, but it was given equally to both groups. However, it appears to have no mechanical effect on rabbit tissues, as evidenced by the work of Moorman et al. [25] which states that there was no statistically significant difference in the values of mechanical properties of biological tissues (tendons) from rabbits treated with ibuprofen versus those treated with placebo at either 14 or 28 days after injury. The use of steroids as a means to prevent stricture formation and adhesions is controversial. A meta-analysis of several studies in humans has shown no benefit (and possible harm) in the use of steroids in caustic esophageal burns, as higher stricture develops, masking clinical perforation and raising the risk of secondary infection and mortality. Conclusive studies demonstrating the beneficial effects of steroids on stricture formation were not found in the literature. Therefore, steroids were not used in this study [26].

A limitation of this study is that it does not objectively measure postoperative pain, but daily clinical monitoring showed no significant differences between groups.

The results of this animal model showed a significantly lower Stenosis Index in the treated group. The fact that the thickness of the esophageal wall in both groups has remained constant, while the luminal diameter has not, supports the hypothesis that the device does not increase tissue damage and allows the preservation of the intraluminal space. This tube's protective effect against stricture formation could be explained by the reduction of potential inflammation, and fibroblast collagen secretion, which improved the organization into fine bundles, allowing reparation with less formation of scar tissue [27,28]. These findings will need to be confirmed in children with caustic burns of the esophagus, who are ultimately the main beneficiaries of this translational research.

In conclusion, the results of this study show that early intraesophageal tubing is an effective procedure for the improvement of the healing process of caustic burns, because it decreases the Stenosis Index and does not produce greater tissue damage in this animal model.

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