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Preliminary study of efficacy of hyaluronic acid on caustic esophageal burns in an experimental rat model

Muazez Cevik[a,*], Tuncer Demir[b], Cetin Ali Karadag[c], Muzaffer Aydin Ketani[d], Hakim Celik[e], Davut Sinan Kaplan[b], Mehmet Emin Boleken[a]

[a]*Faculty of Medicine, Department of Pediatric Surgery, Harran University, Sanliurfa, Turkey*
[b]*Faculty of Medicine, Department of Physilogy, Gaziantep University, Gaziantep, Turkey*
[c]*Department of Pediatric Surgery, Sisli Etfal Training and Education Hospital, Istanbul, Turkey*
[d]*Faculty of Veterinary, Department of Histology and Embryology, Dıcle University, Diyarbakir, Turkey*
[e]*Faculty of Medicine, Department of Clinical Biochemistry, Harran University, Sanliurfa, Turkey*

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Abstract

Background: The aim of this study was to investigate the effectiveness of hyaluronic acid on the prevention of esophageal damage and stricture formation after experimental caustic (alkaline) esophageal injury in rats.

Materials and Methods: Twenty-one Wistar albino rats were randomly divided into three groups. A caustic esophageal burn was created following the Gehanno model: Group l ($n=7$) underwent operation, but no injury; Group 2 ($n=7$) was injured and left untreated; and Group 3 ($n=7$) was injured and treated with hyaluronic acid, first topically and then orally by gavage (2×0.3 mL; 12.5 mg/mL for 7 days). The caustic esophageal burn was created by instilling 25% NaOH into the distal esophagus. All rats were euthanized on day 22 for evaluation. The efficacy of hyaluronic acid treatment was assessed histopathologically and biochemically via blood determination of the total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), and sulfhydryl group (SH) and lipid hydroperoxidase (LOOH) levels. Statistical analyses were performed.

Results: Weight gain was significantly lower in Group 2 than in the other two groups ($P<0.05$). The mean stenosis index, histopathologic damage score, TAS, TOS, OSI, and SH and LOOH levels were higher in Group 2 than in the other two groups. The mean stenosis index, inflammation, TAS, SH and OSI in Group 2 were significantly different than those in the other two groups ($P<0.05$).

Conclusion: Hyaluronic acid treatment is effective in treating damage and preventing strictures after caustic esophageal burn in rats.

The ingestion of caustic substances is a health care problem of the industrial age. It is a pediatric emergency with serious sequelae worldwide and usually occurs by accidental exposure of children less than 6 years of age [1-4]. Children of this age are curious; they are able to drink without

* Corresponding author. Harran Universitesi Tip Fakultesi, Cocuk Cerrahisi Anabilim Dali, Morfoloji Binasi, Yenisehir Kampusu, TR-63300, Sanliurfa, Turkey. Tel.: +90 414 312 84 56 3420; fax: +90 414 313 96 15.
E-mail address: cevikmuazzez@gmail.com (M. Cevik).

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assistance, but are unable to discriminate nontoxic from toxic liquids [2].

Most caustic injuries are superficial and heal spontaneously. Approximately 7% develop into severe burns and lead to stricture at the injury site as a late complication [1,2]. The main aim of treatment is to avoid stricture; however, treatment may pose a challenge. Many previous experimental studies have suggested several types of treatment procedures to prevent the development of stricture [1,2]. They may not be used clinically, however, because of toxicity, cost, or availability problems, and a substantial number of these substances have not been proven reliable in humans. Mitomycin C has been used to treat esophageal strictures in animal and clinical studies. These studies suggest that mitomycin C prevents scar formation but long term outcomes are not available. Mitomycin C may potentially cause a secondary malignancy in the long term. In vivo studies show that mitomycin may induce chromosomal aberrations [1].

Hyaluronic acid is used in many clinical situations as diverse as neurosurgery and cutaneous wound healing [5]. It is a natural polysaccharide (glycosaminoglycan composed of repeated disaccharides of D-glucuronic acid and *N*-acetylglucosamine [6]) in the extracellular matrix of living tissue, such as connective and epithelial tissue [6]. Aqueous solutions of hyaluronic acid are used as a lubricant and cytoprotective agent; it has a high capacity for water absorption and, more importantly, is hypoallergenic, easy to administer, has natural antibacterial properties, and no systemic side effects [6].

We found no biochemical or histopathologic studies in the literature on the effects of hyaluronic acid on wound healing or late-onset stricture formation in clinical or experimental research on caustic esophageal burns; however, it has been used for urethral stenosis [7]. Under certain conditions, increases in oxidants and decreases in antioxidants cannot be prevented, and disturbances in the oxidant/antioxidant balance are considered a causative factor underlying oxidative damage to cellular molecules.

Our study aimed to answer three specific questions:

(1) Is hyaluronic acid an effective pharmacological agent for wound healing in caustic esophageal injury?
(2) Can hyaluronic acid prevent stricture?
(3) Is there any relationship between the treatment outcome and the oxidative stress index in the blood in caustic esophageal burn?

1. Materials and methods

1.1. Experimental animals and drug preparation

This study was approved by the Animal Ethics Committee of the Medical Faculty, University of Gaziantep and conformed to the Guide for the Care and Use of Laboratory Animals (U.S. National Institutes of Health Publication no. 85-23, revised 1996).

Twenty-one Wistar albino rats weighing 153 to 170 g were used for the study. All rats were kept in conditions of 22 °C±2 °C, with a controlled light cycle (12-h day, 12-h night). The animals were fed a standard liquid form of raw chow and water ad libitum.

Animals were treated with hyaluronic acid (hyaluronic acid sodium salt from *Streptococcus equi*; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in0.9% NaCl. Specifically, a solution of 12.5 mg/mL was prepared.

1.2. Experimental study design

Wistar albino rats (n=21) were randomly allocated to three groups as follows:

Group 1 (operated) (n=7): according to the experimental protocol, the rats underwent instillation of 0.9% NaCl, which continued at 0.3 mL of 0.9% NaCl twice a day for 7 consecutive days via gavage.
Group 2 (injured) (n=7): according to the experimental protocol, all stages of the experiment were applied to this group. However, no treatment was applied and the rats received 0.3 mL of 0.9% NaCl twice a day for 7 consecutive days via gavage.
Group 3 (treated) (n=7): all stages of the experimental protocol were applied and 0.3 mL of 12.5 mg/mL of hyaluronic acid was administered to the distal segment of the esophagus for 180 s. The same dosage was continued twice a day for 7 days by oral gavage.

1.3. Caustic esophageal burn

At the beginning of the study, all rats were weighed before the procedure. The rats were fasted for 3 h prior to the procedure and kept under observation while their stomachs emptied. Anesthesia was administered intraperitoneally with 100 mg/kg ketamine (Ketalar; Pfizer, Istanbul, Turkey) and 15 mg/kg of 2% xylazine hydrochloride (Rompun; Bayer, Istanbul, Turkey). The method described by Gehanno and Guedon [8] in 1981 was used to create standard caustic esophgeal burns. The esophageal injury was created by instilling a 25% NaOH solution 0.3 mL volume for 60 s. The abdominal area was shaved, and the surgical site was cleaned. The rats were placed in a supine position on an operating board angled at 30° to reduce the risk of aspiration. The skin of the anterior wall of the abdomen was cleaned with 10% povidone iodine (Poviiodeks; Kimpa, Istanbul, Turkey) from the xiphoid to the symphysis pubis, including both lateral rectus muscles. After ensuring sterile surgical conditions, a 3-cm midline laparotomy incision was performed starting immediately below the xiphoid. The stomach was mobilized through the incision, and the distal section of the esophagus together with the cardia was freed from the surrounding tissues. The cardia together with a 1- to 1.5-cm abdominal esophageal segment was isolated and tied with 3-0 silk sutures proximally to avoid reflux. The anterior