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THE EFFECT OF EARLY FIBROBRONCHOSCOPIC SANITATION WITH HUMAN COLLAGEN TYPE 1 ON EPITHELIALIZATION OF DAMAGED TRACHEAL AND BRONCHIAL MUCOSA IN PATIENTS WITH INHALATION INJURY

A.V. Makarov*, A.V. Mironov, I.Y. Galankina, S.V. Smirnov, I.N. Ponomaryov, N.V. Borovkova

Department for Urgent Endoscopic Studies

N.V. Sklifosovsky Research Institute for Emergency Medicine of the Moscow Healthcare Department
Bolshaya Sukharevskaya Square, 3, Moscow 129090, Russian Federation

* **Contacts:** Aleksey V. Makarov, endoscopist of the Department for Urgent Endoscopic Studies, N.V. Sklifosovsky Research Institute for Emergency Medicine. E-mail: AVMakarov21021@mail.ru

BACKGROUND Inhalation trauma remains one of the most common and complex condition to be treated. Fibrobronchoscopic sanitation plays a significant role in the complex therapy of patients with inhalation trauma, allowing purulent necrotic discharge, soot and combustion products to be removed from the lumen of the tracheobronchial tree. We proposed a method for local treatment of mucosal lesions in inhalation trauma based on early endoscopic sanitation of the tracheobronchial tree with solutions of antiseptics followed by application of human type 1 collagen solution.

AIM OF STUDY To assess the effect of early fibrobronchoscopic sanitation with application of human-type collagen 1 on the timing of epithelialization of the damaged tracheal and bronchial mucosa in patients with inhalation trauma.

MATERIAL AND METHODS The study included 59 patients with inhalation trauma of 2–3 degree. All patients from the first day after the trauma underwent bronchoscopic sanitation in accordance with the standards of medical care. Immediately after the removal of soot, the solution of human-type collagen 1 was applied in patients of the main group, obtained from ligaments and tendons by the acid extraction method. The dynamics of the reparative process was evaluated on the basis of the endoscopic study and according to a series of morphological studies of the biopsy material obtained in the course of fibrobronchoscopy.

RESULTS Complete epithelialization of erosions of the mucous membrane of the trachea and bronchi in patients with inhalation trauma of 2 degree occurred significantly earlier (3 (2; 6) days) than without its application (7 (4; 9) day) ($n_1=15$; $n_2=21$; $U=49.5$; $p=0.0004$). In patients with inhalation trauma of 3 degree, epithelialization time was reduced from 17 (12; 22) days in the comparison group to 7 (6; 9) days in the main group ($n_1=14$; $n_2=9$; $U=1$; $p=0.001$). According to the morphological study, a characteristic feature of the fibrobronchoscopic sanitation with the human collagen type 1 was the absence of purulent inflammation of the trachea and bronchial wall.

CONCLUSION In the study, it was statistically proved that the early fibrobronchoscopic sanitation and application of the human collagen type 1 solution for acute lesions of the mucous membrane of the trachea and bronchi twice accelerates epithelialization of mucosal lesions without the development of purulent inflammation.

Keywords: inhalation trauma, fibrobronchoscopy, mucosal damage, human type 1 collagen

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ORCID

A.V. Makarov: 0000-0001-7388-7077

I.N. Ponomaryov: 0000-0002-2325-6939

N.V. Borovkova: 0000-0002-8897-7523

FBSS – fibrobronchoscopic sanitation

IT – inhalation trauma

TBT – tracheobronchial tree

Among burn injuries of patients admitted to the Burn Center of the N.V. Sklifosovsky Research Institute for Emergency Medicine, the proportion of inhalation trauma (IT) was 17.5% in 2014, and 14.3% in 2015. At the same time, international statistics indicate a trend towards its steady growth [1, 2]. In case of isolated IT, the mortality rate may reach 23.0–27.6% [3–5].

Specialists consider the complex effect of high temperature inhaled air, soot and combustion products on the mucous membrane of the lower respiratory tract as the key pathogenesis component of IT. The latter, interacting with the secretion of the trachea and bronchi, form strong alkalis and acids, which cause a chemical burn in situ. In this case, the soot does not have a direct damaging effect on the mucous membrane, but due to the transfer of toxic compounds contributes to the growth of the lesion area. As a result, erosive and ulcerative defects are formed on the mucosa, and the necrotized epithelium, together with soot, mucus and fibrin, forms dense conglomerates that enclose the lumen of the bronchi. The disturbance of ventilation leads to atelectasis in the lungs and the development of pneumonia, which increases the severity of the trauma [6, 7].

The treatment of patients with IT includes a wide range of measures aimed at management of homeostasis, preventing infectious complications and providing conditions for regeneration of the mucous membrane of the respiratory tract. One of the main methods of treatment is fibrobronchoscopic sanitation (FBSS). It is necessary to perform it in the early days

after trauma, since it not only promptly determines the depth and extent of the lesion, but also allows to remove the substrate for the development of purulent inflammation (combustion products, necrotic masses), and to reduce the incidence of infectious inflammation as well [8, 9].

Currently, a number of modifications of the standard FBSS procedure have been suggested, which can contribute to mucosal recovery [10]. However, they were not widely spread due to the complexity or inherent shortcomings. Thus, the prevalence and severity of the course of IT in patients with thermal lesions makes it extremely urgent to develop relatively simple and safe methods of local endoscopic treatment that will help restore the mucosa of the trachea and bronchi.

In combustiology, wound coatings based on collagen are successfully used to shorten epithelization terms of 2-3A skin burns [11]. The clinical effect of this fibrous protein is achieved by ensuring the migration of epithelial cells to the area of damage. Proceeding from this, it can be assumed that application of collagen to the areas of mucosal lesion during FBSS will also contribute to restoration of its integrity. However, this theory certainly requires confirmation.

The aim of the study was to evaluate the influence of early FBSS with application of human type I collagen on epithelization of lesions of the mucous membrane of the trachea and bronchi in patients with IT.

MATERIAL AND METHODS

The study was performed on the basis of the Department for Acute Thermal Lesions, Department for Urgent Endoscopic Studies, Laboratory of Cell Transplantation and Immune Typing and Pathological Anatomy Unit of the N.V. Sklifosovsky Research Institute for Emergency Medicine with the approval of the Ethics Committee and Scientific Council of the Institute.

The open-label, randomized, prospective study included 59 patients with IT grade 2-3 who were on treatment at the Burn Center from 2014 to 2017. The main group included 29 people (12 men and 17 women) of 20-89 years, median — 55.5 (35.7, 69.0). In 15 cases, IT of grade 2 was diagnosed, and in 14 cases IT of grade 3 was diagnosed. The comparison group included 30 patients (20 men and 10 women) of 26-90 years: median — 58.5 (44.5, 74.5). The severity of IT in 21 cases corresponded to grade 2, and in 9 cases to grade 3. Thus, the studied groups were similar in the number of patients, gender, age and severity of the respiratory tract injury.

FBSS was performed daily, starting from the first day after the injury in all patients as a component of treatment corresponding to the severity of trauma. The procedures were carried out according to a standard technique through the natural airway (under local anaesthesia) or via the intubation tube (under an intravenous sedation) using flexible bronchoscopes *OLYMPUS BF 1T60* or *Q180*. During sanitation, the necrotic detritus and soot from the trachea and bronchi were removed with a 0.01% solution of Dioxidine or Miramistin. In patients of the main group, a solution of human collagen type I was additionally applied to the mucosal lesions after removal of soot. The volume of the drug used, depending on the severity and extent of damage, was 2-4 ml. On day 2-3 after application of collagen in the absence of visual endoscopic signs of epithelial damage, the treatment procedure was repeated, but no more than 3 times.

A specially prepared solution of human type I collagen was used. Collagen was obtained from ligaments and tendons by the method of acid extraction in the Tissue Preservation and Transplantation Department of the N.V. Sklifosovsky Research Institute for Emergency Medicine. All the initial biomaterial corresponded to the criteria of infectious and toxicological safety. The collagen solution was prepared in accordance with the previously developed method (RF patent for invention no. *RU 2591544 C1*). To ensure passage through the endoscope channel, collagen was diluted with an aqueous 0.01% chlorhexidine solution. To ensure the possibility of controlling the distribution and fixation of the drug, it was stained with a 0.2% aqueous solution of a brilliant green. The resulting composition was sterilized with ultraviolet rays for 60 minutes.

The dynamics of the course of the wound process was evaluated with repeated FBSS. Endoscopic criteria confirming the positive dynamics of the reparative process were decreased hyperaemia and edema of the mucous membrane, a decrease in the amount of fibrin overlays, fragmentation and reduction in lesion size, and the appearance of focal-thickened sections of the mucosa at the edges of ulcerous defects.

For a detailed study of the process of repair of acute thermochemical damage to the mucous membrane of the trachea and bronchi, a series of biopsy specimens from the edges of defects were investigated using a human-type collagen solution. The biomaterial was fixed with 70% ethyl alcohol, after which it was subjected to a standard procedure for manufacturing paraffin histological preparations. Staining of the finished microsections was made with hematoxylin and eosin, PAS reaction, and according to Van Gieson.

The statistical processing of the data was carried out with the determination of the median and interquartile range. The statistical significance of the differences in the same indices between unbound samples was determined using the Mann-Whitney U test.

RESULTS

During the primary FBSS in all patients with IT grade 2, a large amount of mucous secretion with an admixture of soot was found in the lumen of the trachea and bronchi. On the walls of the respiratory tract there were confluent thin soot coatings, which were partially removed during the sanitation. Moreover, the mucous membrane, freed from soot, was hyperemic, edematous and covered with acute erosions. In cases of IT grade 3, the discharge had a thick consistency with a lot of soot or was absent in the lumen of the respiratory tract. There were massive confluent overlays of dense soot on the

walls of the trachea and bronchi adhering to the underlying tissues through the intima. The mucous membrane was easily vulnerable, bleeding, pale gray, with mild edema or without it, covered with both erosions and ulcers.

The application of the collagen solution was performed after complete cleansing of the mucous membrane of the tracheobronchial tree (TBT) from the soot. In the patients of the main group with IT grade 2, the damage to the mucous membrane was covered with the collagen solution already at the first sanitation, and in cases of IT grade 3 – on day 2-3 after the trauma. When endoscopically applied, the collagen solution was independently distributed and fixed in the form of a thin film solely in the places of damage, including the underlying ones. Due to the dye included into the solution, the collagen distribution was clearly visualized on the surface of defects. There were no complications during this procedure.

Further observation showed that in the patients of the main group with IT of grade 2, the severity of the edema and hyperemia of the mucous membrane of TBT decreased on day 1-2 after the first application of collagen, and acute erosions decreased in size, which indicated the beginning of defects' epithelialization. In case of IT of grade 3, the endoscopic signs of inflammation regressed somewhat later, on day 2-3. Ulcerous defects were fragmented, decreased in size, and their edges acquired a clear contour due to areas of thickening of the epithelium. According to histological data, early appearance of lymphocytes and macrophages was noted in the patients of the main group after application of the collagen solution on erosion and ulcers in the surrounding tissues. The activation of fibroblastic reaction occurred already on day 4-5, which was observed at a later date in case of standard FBSS. In this case, the layers of multirowed epithelium intensively moved from the edges of the defects along the allogeneic collagen. Complete epithelialization of mucosal lesions in the main group of patients (collagen solution application) with IT of grade 2 was observed on day 2-6 (Fig. 1), and on day 6-9 in case of IT of grade 3 (Fig. 3).

In contrast, in the patients of the comparison group, the mucous membrane of TBT remained edematous and brightly hyperemic, the lesion size remained the same, and their surface was covered with massive fibrin overlays until the appearance of the first signs of epithelialization. The first endoscopic signs of epithelialization of mucosal in IT grade 2 were seen only on day 4-5 after trauma, and on day 9-13 after IT of grade 3. In the histological study of biopsy specimens, the dynamics of the wound process was characterized by a prolonged preservation of foci of fixing soot, fibrin on the bottom of defects with severe purulent inflammation and infiltration by polymorphonuclear leukocytes. Complete epithelization of damage to the mucous membrane of TBT against the background of standard FBSS in patients with IT of grade 2 was noted on day 4-9 (Fig. 2), and on day 12-22 in case of IT of grade 3 (Fig. 4).

Thus, in the case of local application of collagen, complete epithelization of erosions of the mucous membrane of the trachea and bronchi after IT of grade 2 occurred statistically significantly earlier (3 (2; 6)) than without its application (7 (4; 9)) ($n_1=15$; $n_2=21$; $U=49.5$; $p=0.0004$). In IT of grade 3, covering of the ulcers of the mucous membrane with a collagen solution also statistically significantly reduced epithelization time for them on average to 7 (6; 9) days, while after the standard procedure of FBSS this period was about 17 (12, 22) days ($n_1=14$; $n_2=9$; $U=1$; $p=0.0001$).

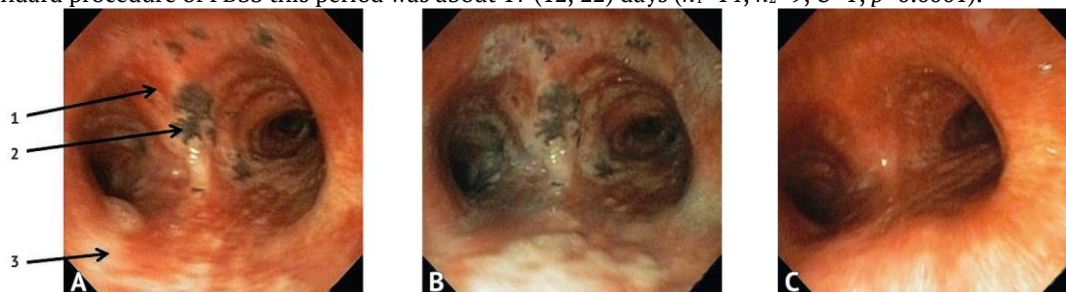


Fig. 1. The endoscopic view of wound process in case of inhalation trauma of the 2nd degree when collagen type I is applied. A – 2 days after the injury: 1 – acute erosion; 2 – foci of fixed soot; 3 – foci of light fibrin in the mucosa of the trachea and bronchi. B – application of collagen type I solution. C – 3 days after application of collagen solution: complete epithelialization of erosion of the mucous membrane

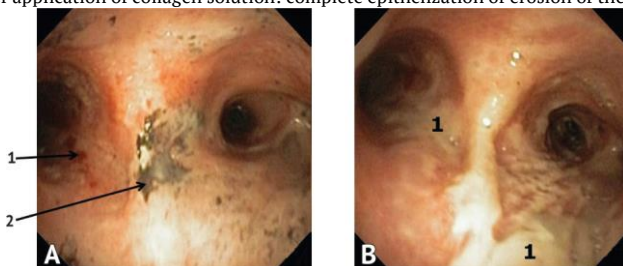


Fig. 2. The endoscopic view of the wound after inhalation trauma of the 2nd degree without application of human collagen type I. A – 1 day after injury: 1 – acute erosion; 2 – foci of fixed soot. B – 4 days after the trauma: 1 – confluent fibrinous foci on the mucous membrane of the trachea and bronchi

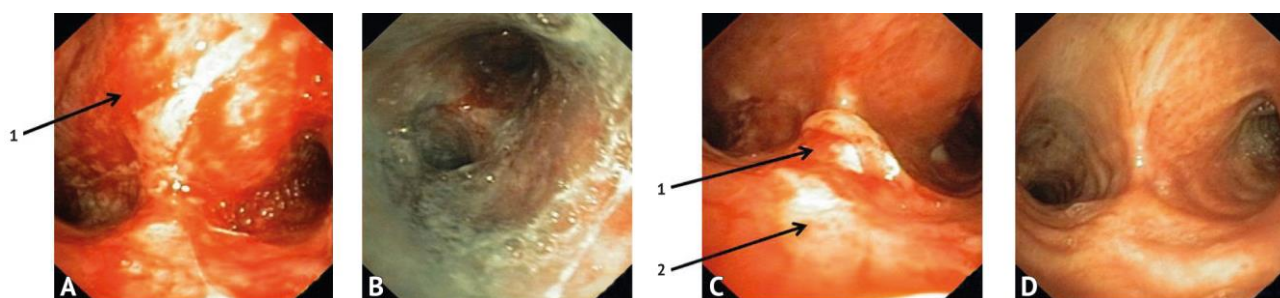


Fig. 3. The endoscopic view of the wound after inhalation trauma of the 3rd degree when using collagen type I. A — 2 days after the trauma: 1 — confluent ulcers after the removal of soot without clear contours. B — application of human collagen type I solution. C — 4 days after the application of collagen type I: 1 — ulcer-like defects were fragmented, their edges became clear and even; 2 — layers of thickened epithelium at the edges of ulcerative defects. D — 10 days after application of collagen type I: complete epithelialization of ulcer-like mucosal defects

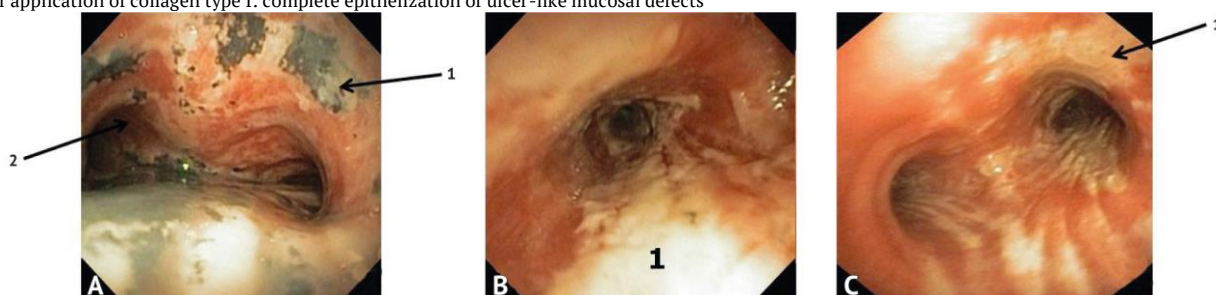


Fig. 4. The endoscopic view of the wound after inhalation trauma of the 3rd degree without application of human collagen type I. A — 1 day after the injury: 1 — confluent overlays of tightly fixed soot; 2 — superficial ulcerous defects without clear contours. B — 5 days after injury: 1 — confluent overlays of light thick loose fibrin. C — 12 days after injury: the endoscopic view without significant dynamics: 1 — confluent overlays of light thick loose fibrin

CONCLUSION

Inhalation trauma is one of the most common and difficult pathological conditions to be treated. In this case, the combination of the respiratory system lesion with skin burns leads to the development of a syndrome of mutual confounding effects, which aggravates the burn shock and increases the mortality rate [9]. Specialists consider fibrobronchoscopic sanitation as an essential part of the complex therapy for patients with inhalation trauma. It is a reliable, relatively simple method of diagnosis and provides the ability to perform therapeutic manipulations.

We have presented a method for local treatment of mucosal lesions in inhalation trauma based on early thorough endoscopic lavage of the lumen of the tracheobronchial tree with solutions of antiseptics followed by application of human type I collagen solution onto the clean bottom of defects. With its use, complete epithelialization of erosions occurred averagely on day 3, and epithelialization of ulcers occurred on day 7 (standard treatment 7 and 17 days, respectively). In the histological analysis of biopsies from the bottom of lesions, the appearance of lymphocytes and macrophages, especially around the collagen solution, was noted in the patients of the main group after the first application. After IT of grade 3, the activation of the fibroblastic reaction and the appearance of multirowed epithelium layers "moving" over the collagen covering the bottom of the defect occurred on day 4-5. The main distinguishing feature characteristic of cases of early endoscopic sanitation bronchoscopy with application of human type I collagen solution at different stages of the wound process was absence of purulent inflammation of the trachea and bronchial wall, as well as early activation of epithelial growth in the edges of defects. At the same time, in patients of the comparison group, the course of the wound process was usually accompanied by the development of severe purulent inflammation with extensive burn wounds regeneration of grade IIIA with the use of bandages with collagen type I [11].

Thus, early fibrobronchoscopic sanitation and application of human type I collagen to acute lesions of the mucous membrane of the trachea and bronchi, resulting from inhalation trauma, prevent the development of purulent inflammation in situ and promote acceleration of epithelialization twice.

FINDINGS

1. Early fibroblochosopic sanitation and application of the human type I collagen solution to acute lesions of the mucous membrane of the trachea and bronchi allow to shorten the periods for complete epithelialization of erosions (IT of grade 2) and ulcers (IT of grade 3) by 2,3-2,4 times from 7 (4, 9) to 3 (2, 6) days ($n_1=15$; $n_2=21$; $U=49.5$; $p=0.0004$) and from 17 (12; 22) to 7 (6; 9) days ($n_1=14$; $n_2=9$; $U=1$; $p=0.0001$), respectively.

2. Early fibrobronchoscopic sanitation and application of human type I collagen solution prevents the development of purulent inflammation in situ.

3. Epithelialization of lesions with the use of human type I collagen solution occurs due to activation of fibroblastic reaction and appearance of multirowed epithelium layers "moving" along the allogeneic collagen covering the defect.

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