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Short communication

Differential scanning calorimetric examination of the tracheal cartilage after primary reconstruction with continuos sutures A preliminary study

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Abstract

Acquired upper airway stenosis is usually associated with a complex of pathological conditions at the high tracheal and the subglottic levels. Reported reconstructive techniques include widening of the airways by incorporation of grafts, segmental resection, and anastomosis or combined procedures. Progress in anaesthesia, surgical techniques, and understanding of the pathophysiology of the trachea has made primary tracheal reconstruction a safe operative procedure, although there are no reports observing its acute effect on the tracheal cartilage. Differential scanning calorimetry (DSC) is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems, including hyaline cartilage, but it has never been applied for the investigation of tracheal cartilage. According to the present study, the thermograms may prove the presence of structural changes of the cartilage after primary reconstruction in the short-term follow up (smaller melting temperature and calorimetric enthalpy in the operated dog). The differences were clearly demonstrated between the intact cartilages and the ones involved in the anastomosis.

Keywords: DSC; Tracheal cartilage; End-to-end anastomosis; Continuous sutures

1. Introduction

Resection and subsequent end-to-end anastomosis of the windpipe is a tried-and-tested acceptable method for the surgical treatment of small segmental defects of different etiology [1]. Various forms of these pathologies may develop after traumatic injury, chronic inflammation, long-term intubation, benign and malignant tumors. The firm and unquestionable statement of H.C. Grillo is well accepted among physicians that the resection is possible, only if the length of injury does not exceed the 50% of the total trachea in adults and 30% in infants [2].

There are a variety of different techniques for tracheal end-to-end anastomosis, with the modification of the tracheal resection technique, suturing type and the applied suture material [3–7].

Relatively high incidence of postoperative complications [8] highlight the fact that the suturing technique in the anastomosis is still subject to debate. Continuous suturing technique seems to offer a suitable method for the reconciliation of the tracheal stumps, as it provides a more event distribution of tension around the tracheal lumen, and an increase in the tensile strength [4]. Other authors emphasize the beneficial postoperative effect of the interrupted suturing technique [7]. To our knowledge investigators dealing with this very problem have only indirect evidence at hand based on postoperative results. We aimed to show the intra-operative effect of the continuous suturing technique on the microcirculation as well as the late postoperative changes of the tracheal cartilage.

2. Hypothesis-objectives

Our hypothesis was that after resection of the windpipe and completing the primary end-to-end anastomosis there is a decrease in the local microcirculation resulting in de-

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formation of the tissue elements building up the cartilage. We examined cartilages involved in the tracheal anastomosis during primary reconstruction in contrast to the healthy cartilage.

Our aim was to prove with the examinations that there is a definitive difference in the structure of the healthy cartilage, and that of involved in the anastomosis.

Objectives of research were:

1. Introduction of the application of a new method in tracheal research.
2. Setting up of calorimetric standards of normal tracheal cartilage.
3. Applying calorimetric methods for the investigation of cartilage involved in the anastomosis.

Presentation of the differences in the samples of normal and changed conditions.

3. Materials and methods

3.1. Animal preparation and anaesthesia

All experiments were in accordance to rules and regulations regarding the use of animals in medical research. The study was approved by the local authorities (BA02/2000-16/2001). Seven healthy adult beagle dogs (four ♀, three ♂, 12–19 kg) were randomly selected and denied access to food 6 h prior to surgery. Droperidol (1.5 mg/kg), fentanyl (0.03 mg/kg) and atropin (1 mg). Anaesthesia was induced with i.v. Thiopental-sodium (5–10 mg/kg) and maintained with 0.5% halothane in 70% nitrous oxide and 30% oxygen gas mixture. Lidocaine hydrochloride was injected at the operative site pre- and postoperatively.

3.2. Surgical procedure

Following a midline neck incision the neck muscles were separated to circumferentially expose the trachea below the larynx. Resection was performed with surgical blade between the third and fourth tracheal cartilage. Haemostasis was achieved with bipolar coagulation and the use of haemostatic sponge (Surgicel, Johnson & Johnson, New Brunswick, NJ). After completing the resection, ventilation was maintained with a sterile tracheal tube through the distal stub. We used continuous suture technique in every case (PDS, 4/0, Ethicon Inc., Somerville, NJ). Sutures were tension-free and traversed the tracheal ends in full thickness, incorporating one tracheal ring at each end.

After completing the anastomosis operative area was closed according the appropriate surgical rules.

The animals were sedated and painlessly euthanized after a 20 days of follow up.

3.3. Laser Doppler measurements

Laser Doppler measurements (MBFD3, Moor Instruments England) were performed anteriorly at three different points on the first proximal and distal tracheal rings related to the resection lines, and on the eighth tracheal ring, which served as control in the experiments. Triple measurements were taken before and after the resection and following completion of the anastomoses. An adjustable metal platform was designed to maintain a stable position of the Laser Doppler probe above the operative field.

3.4. Sample preparation

After the follow up period rings of the anastomotic area and the control were removed, and carefully derived from tissue fragments taken during the operation and considered to be waste material.

All the cartilage samples weighted identically ca. 100 mg, which represents a 5 mm long, 5 mm wide segment with a height of 3 mm. Samples were washed three times in PBS (sterile phosphate-buffered saline, pH 7.4) in order to eliminate all extracartilaginal tissue remnants. Samples were then put into RPMI-1640 solution (SIGMA) containing 10% fetal bovine serum (HYCLONE laboratories), antibiotic, antimycotic solution (1 U/ml penicilline, streptomycine, gentamycine and fungisone, GIBCO lab), non-essential amino acids (GIBCO) and sodium carbonate. All the individual samples were stored separately at 4 °C, no longer than 24 h. Then samples were subjected to calorimetric measurement.

3.5. DSC measurements

The thermal unfolding of healthy and operated trachea cartilage was monitored by a SETARAM Micro DSC-II calorimeter. All experiments were carried out between 0 and 100 °C with a scanning rate of 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with an average 850 μl sample volume. RPMI-1640 buffer was used as a reference sample. The sample and reference vessels were equilibrated with a precision of 0.1 mg. It was not necessary to correct for heat capacity between the sample and reference vessels. The calorimetric enthalpy was calculated by the SETARAM two points fitting integrating software.

3.6. Statistical analysis

Results are expressed as mean values ± S.E.M. Microcirculation data were analyzed with 1-way analysis of variance (ANOVA). The level of significance was set at $P < 0.05$. The Micro Cal Origin (ver. 6.0) program (Microcal Software Inc., Northampton, USA) was used for graphical presentation.

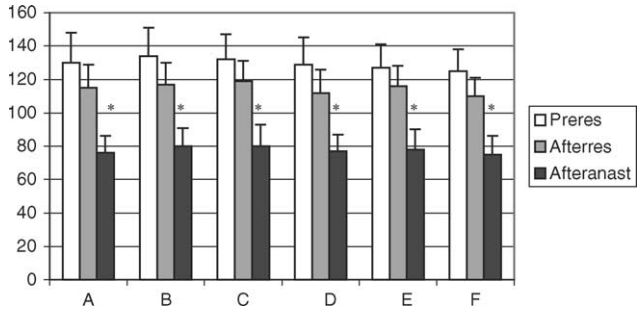

Fig. 1. The microcirculatory data before (□) and following (▨) the tracheal resection and after completing the anastomosis (■). Significant decrease was seen in each points (A–F) of the operated area ($^{*}P < 0.05$).

4. Results and discussion

4.1. Microcirculation

Fig. 1 demonstrates alterations in microcirculatory values. Results depict no change after the resection of the trachea, but significant decrease following the completion the anastomosis with continuous sutures. No effect was seen on the control tracheal rings.

4.2. DSC measurements

Tracheal rings are built up of hyaline cartilage, which forms a supporting framework to resist the pressure changes in the airways. It is composed of chondrocytae, cartilage matrix (collagen types II, IX, X, XI), proteins of non-collagen type (proteoglycans), inorganic materials, and water. Collagen fibres are responsible for the tensile strength of the tracheal cartilage, while proteoglycans are responsible for compressibility [9]. Than et al. demonstrated that DSC is a useful method for the investigation of hyaline cartilage in different stages of osteoarthritis [10]. They explained the pronounced heat capacity change between intact and arthritic samples with the structural alterations in osteoarthritis caused by the biochemical processes.

Effect of ischaemia on the tracheal cartilage is not perfectly understood, as conventional histologycal examination can not show any marked difference of acute cartilage damage. In our study, we could demonstrate the ischaemic effect of tracheal sutures on the microcirculation of the trachea. Following structural change was not demonstrable by conventional hematoxylin eosin (HE) staining. The effect of long-term ischemia is however well documented in experimental models and clinical studies following prolonged airway intubation [11]. It results in the clinical picture of tracheomalacia, airway weakening, which has marked structural change of the cartilagenous structure of the trachea [12,13]. With the use of continuos sutures we could demonstrate the subnormal circulation of the trachea, which may cause a trigger towards tracheomalacia. This is supported by the clinical observation, that after airway

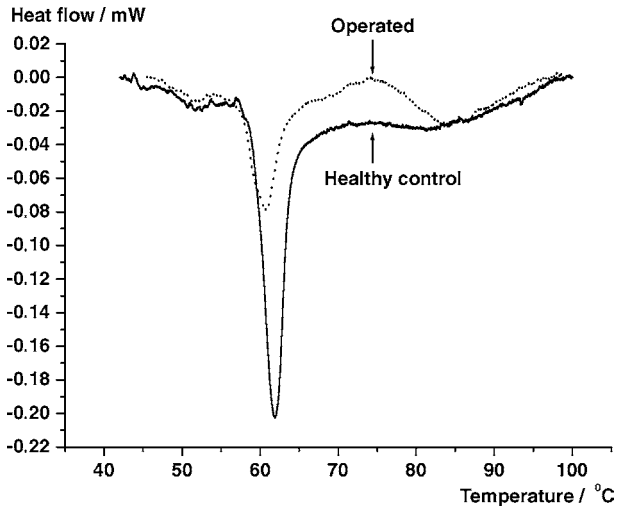

Fig. 2. Thermal denaturation of healthy and operated (anastomosis with continuous sutures) dog trachea.

reconstruction with primary anastomosis tracheomalacia is a common complication [14], but to prove this hypothesis DSC measurements of deformed tracheomalacic cartilage should be the way to go, but the lack of patients of this condition limit our possibilities.

DSC scans of healthy and operated tracheal cartilages showed a low (it is not plotted because we got in both case a noisy graph) and a complex high temperature melting characteristics (Fig. 2) during the first heating. The proper thermal parameters of the main transition in average were $T_m = (61.8 \pm 0.4)\,^{\circ}C$ and $\Delta H = (0.49 \pm 0.02)$ J/g for healthy and $T_m = (60.6 \pm 0.3)\,^{\circ}C$ as well as $\Delta H = (0.55 \pm 0.02)$ J/g for operated samples. The effect of continuous sutures is manifested in the decreased T_m, in the appearing a marked endoterm peak at 83 °C which increased the transition enthalpy by 10% too. The influence of suturation is more pronounced on the first cooling curve (Fig. 3) The high

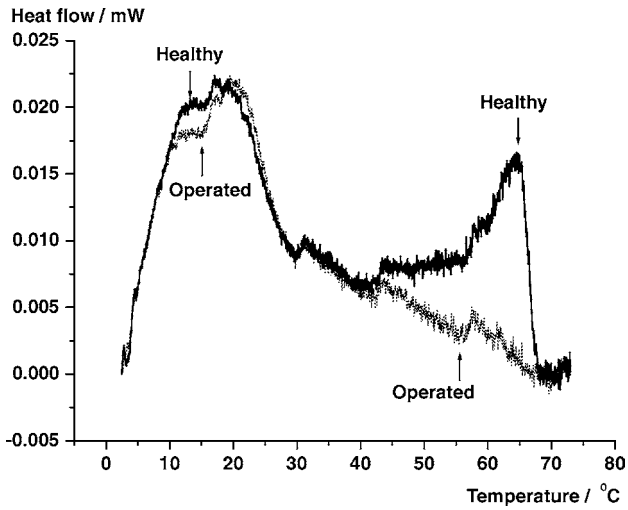

Fig. 3. DSC scans of trachea rings at first cooling.

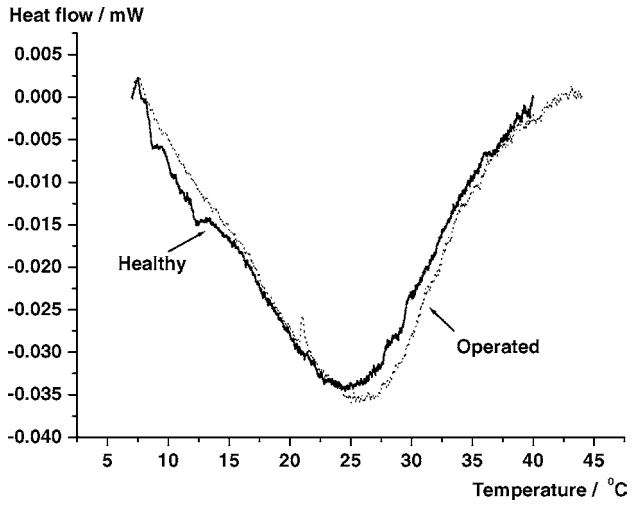

Fig. 4. Reproducibility of low melting transition during first reheating.

temperature melting seems to be reversible for healthy sample in 5% while perfectly disappears for the operated one. The low temperature transition during renaturation is observed in both cases at the same temperature with the same calorimetric enthalpy. It appears during the second reheating (Fig. 4) too in same manner for both sample at around 25 °C with the same enthalpy change.

These DSC results of the sutured tracheal cartilage show an even more marked alteration, than the arthrotic articular cartilage already investigated by our research group. This bodes a presence of a more severe damage in a therapeutic surgical tracheal reconstruction, than a destructive pathologic condition developing in decades. Lack of signs for structural difference by conventional histology in contrast to the presence of the marked thermal differences precipitates the biochemical explanation of this problem obviously needing the complex row of studies, as this aspect of the tracheal research has infinitesimal data collected.

Acknowledgements

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