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DSC EXAMINATION OF THE OESOPHAGUS AFTER TWO DIFFERENT SELF-EXPANDABLE STENTS IMPLANTATION
An experimental study

L. Benkő[1], *J. Danis*[2], *M. Czompo*[2], *R. Hubmann*[2], *Andrea Ferencz*[1], *G. Jancsó*[1], *Z. Szántó*[1], *Annamária Zólyomi*[3], *Franciska Könczöl*[4], *Á. Bellyei*[5], *Erzsébet Rőth*[1] *and D. Lőrinczy*[6*]

[1]Department of Surgical Research and Techniques, Faculty of Medicine, University of Pécs, Kodály Z. str. 20, 7624 Pécs, Hungary
[2]L. Boltzmann Institute for Laparoscopic Surgery, Department of Surgery II., General Teaching Hospital Linz, Krankenhaus str. 9 4020 Linz, Austria
[3]Department of Radiology, Faculty of Medicine, University of Pécs, Szigeti str. 12, 7624 Pécs, Hungary
[4]Institute for Forensic Medicine, Faculty of Medicine, University of Pécs, Szigeti str. 12, 7624 Pécs, Hungary
[5]Department of Orthopedics, Faculty of Medicine, University of Pécs, Szigeti str. 12, 7624 Pécs, Hungary
[6]Institute of Biophysics, Faculty of Medicine, University of Pécs, Szigeti str. 12, 7624 Pécs, Hungary

Massive bleeding from oesophagus varices presents a life threatening complication of liver cirrhosis. No effective method of treatment is available until now, that would guarantee high grade of patient wellness during the conditioning and investigation phase until the definitive treatment could be introduced.

The fact that we have not found any report in the literature about self-expandable metal stents (SEMS) application in acute variceal bleeding had encouraged us to use stents usually used for oesophageal malignancy and furthermore develop a special stent for this individual indication.

The aim of this study was to evaluate the tissue response to oesophagus stent designed for stop acute variceal bleeding in animal experiment in compare with another stent used for iatrogenic treatment of different strictures of the oesophagus. Tissue oxygen saturation (StO_2) measurement was performed before and after the implantation of the stents. Macroscopic and histological investigations of the stented oesophagus segments were observed after 10 days.

Differential scanning calorimeter (DSC) is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems, but it has never been used for the investigation of the oesophagus. According to our results the thermal denaturation of intact oesophagus, its mucosa and muscle fragments revealed significant differences compared to healthy sample in favour of the new stent.

Keywords: *DSC, oesophagus variceal bleeding, portal hypertension, SEMS*

Introduction

Acute oesophagus variceal bleeding presents a life threatening complication of portal hypertension. Mortality after the first bleeding episode is up to 50%. After an initial haemorrhage, the frequency of recurrent bleeding ranges between 30–40% within the subsequent 6 weeks, and more than 40% of the patients die within the first year [1, 2]. Variceal bleeding is now more commonly treated pharmacologically and/or via endoscopic sclerotherapy (ES), or endoscopic band ligation (EBL). In some cases bleeding cannot be stopped despite combination of endoscopic and drug therapy, especially in patients after several procedures of sclerotherapy or band ligation leading to sclerosis of the mucosa [3]. The inflatable balloons still have a place however, even if it is simply used to save time in preparation for more definitive treatment, or in case of persistent and recurrent bleeding despite of previous drug therapy and of course in case of immediate primary acute haemorrhage. This method can cause the pressure necrosis of the oesophagus, after 48–72 h. Patients usually should be intubated and ventilated mechanically to prevent pulmonary infection or aspiration.

Consequently, no effective method of treatment is available until now, which would guarantee high grade of patient wellness during the conditioning and investigation phase until the definitive treatment could be introduced. Therefore we searched an alternative method to compress the bleeding varices. The placement of self-expanding metal stents for palliation of malignant oesophagus strictures and oesophago-tracheal fistulas are effective and safe [4, 5]. The fact that we have not found any report in the literature about SEMS application in acute variceal bleeding had encouraged us to

* Author for correspondence: denes.lorinczy@aok.pte.hu

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

use stents usually used for oesophageal malignancy in an emergency situation of varix bleeding instead of balloon tampon at the emergency ambulance and furthermore develop a special stent for this individual indication. We have followed the consequences of our intervention with histology, oxygen saturation and thermal denaturation measurements. The differential scanning calorimetry (DSC) is a sensitive method to look for the local and global effect of any alteration in biological system as it was demonstrated in our similar earlier works [6–9].

Aim of the study

This study is proved the feasibility of the new stent designed for stop acute variceal bleeding, furthermore the destructive effect to the normal oesophagus. The local pressure of the stents may result a decrease in the local microcirculation resulting in tissue damage. We aimed to demonstrate possible deformations of the tissue elements building up the oesophagus after the implantation of two different stents.

Experimental

Materials and methods

Design of the stents and the delivery systems

The new self-expandable, covered metal stent (stent-1) with the introducer set was designed to be an effective and learnable method in the treatment of the acute phase of oesophageal varix bleeding until the definitive therapy could be introduced. Stent SX-ELLA-Danis® (ELLA-CS Company, Hradec Kralove, Czech Republic) is a nitinol (nickel-titanium) monofilament woven wire mesh stent with flared ends preventing of the migration, and a polyurethane inner coating layer. The stent has a length of 105 mm, a body diameter of 21 mm, and a diameter of throats of 28 mm. The midstent (body of the stent) and the two ends have Pt/Ir radiopaque markers, to keep visible on the X-ray examination after the successful implantation or in fortuitous case of migration. Movable stainless steel wires with Au marker were placed on the two ends ensuring the possibility of position correcting or make easier removing after the management. The SX-ELLA-Danis stent has a special delivery system with active length of 60 cm, and body diameter of 22 French (7.3 mm), that allows a placement without radioscopy or even endoscopic control.

The other fully covered self-expandable stent (stent-2) is the FerX-Ella-Boubella oesophageal stent (diameter 21 mm, length 105 mm), designed primary for the iatrogenic treatment of malignant strictures in the oesophagus. This stent is made of stainless steel that has a great corrosion resistance and good radiopacity, which is increased by radiopaque golden markers. The covering of the stent is made of polyethylene. Delivery system of this stent is equipped with an inflatable balloon for the easier positioning.

Animal preparation and anaesthesia

All experiments were in accordance to rules and regulations regarding the use of animals in medical research. The present study was approved by the local institutional committee on animal research of Pécs University (BA 02/2000-29/2001).

Fourteen adult mongrel dogs (five ♀, nine ♂, 9–32 kg) were randomly selected and denied access to food prior to the procedure. After premedication with Droperidol (1.5 mg kg^{-1}), Fentanyl (0.03 mg kg^{-1}) and Atropin (1 mg), short anaesthesia was induced with intravenous Thiopental-sodium injection. Lidocain spray was sprinkled into the pharynx before the procedure in all animals.

Stent introduction

The experiment was carried out in two groups. In the first group (stent-1), the new self-expandable stents (SX-ELLA-Danis) were introduced into the distal oesophagus of seven mongrel dogs (mean body weight 24 kg). After the correct positioning of the stent with an inflated balloon at the distal end of the introduction set, the stent was released by pulling the sheath back. Delivery system was removed after the procedure. In the second group (stent-2) seven mongrel dogs (with average body mass 26 kg) were undergone the same procedure, using the FerX-ELLA-Boubella stent. After the successful stenting the correct position of the stents was identified with gastroscope and X-ray examination in both groups. A watery consumption food diet was administered from the first postoperative day to reduce the risk of early stent migration. 10 days after the procedure the oesophagus was inspected for mucosal injury due to the local pressure of the stent, and the possible necrosis of the oesophagus wall. Histological examinations and DSC measurements of the oesophagus walls were performed.

StO_2 measurement

Tissue oxygen saturation of the oesophagus was monitored with the Inspectra Tissue Spectrometer-Model 325 by means of a 15 mm light emitting/collection head attached to an optical cable placed into the oesophagus. StO_2 data were recorded before (control) and after the intervention at the proximal end, and the middle part of both stented oesophagus segments in all animals.

Sample preparation

After the follow up period the 15 cm long segment of the oesophagus with the stent and the control normal wall above the involved area were removed, and carefully purified from tissue fragments. Oesophagus samples were derived into mucosal and muscle tissue layer, to investigate separately the differences between the distinct tissue constituents and the whole wall.

All the oesophagus samples weighted identically ca. 100 mg, that represents a 5 mm long, 5 mm wide segment with a height of 3 mm. Samples were washed 3 times in PBS (Sterile Phosphate-buffered saline, pH 7.4) in order to eliminate all tissue remnants. Samples were treated with DMEM-F12 solution (GIBCO lab) containing 10% (FBS) foetal bovine serum (HYCLONE lab), antibiotic, antimycotic solution (1 U/mL penicillin, streptomycin, gentamycine and fungisone, GIBCO), 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.

Statistical analysis

Tissue oxygen saturation data were analysed with one-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.

DSC measurements

The thermal unfolding of the healthy and stented oesophagus preparations were monitored by Setaram Micro DSC-II calorimeter. All experiments were conducted between 0 and 100°C. The heating rate was 0.3 K min^{-1} in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (oesophagus samples plus buffer) in average. Typical sample wet weights for calorimetric experiments were between 200–250 mg. DMEM-F12 (with admixtures) buffer was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference vessels. The buffer/empty sample holder scan was used as baseline reference and subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting Setaram peak integration.

Results

The stents (Fig. 1) could be easily inserted with the special introducer in all cases. There were no bleeding or perforation by the introduction of the stents. Correct position of the stents was observed on X-ray examinations after successful implantation in all animals. Stents were well tolerated based on watery food consumption from the second postoperation day and normal behaviour in both groups.

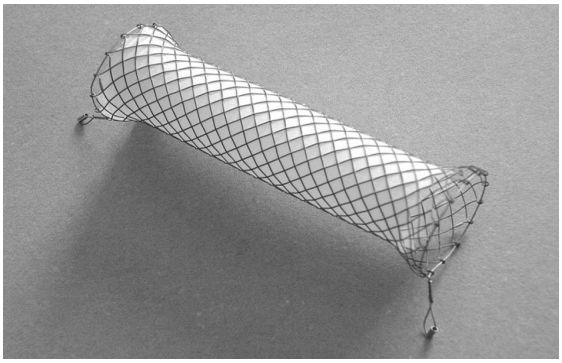

Fig. 1 The new stent: SX-ELLA-Danis (stent-1)

We observed stent migration into the stomach in one case of each group. This caused no perforation or mechanical ileuses and removing could be performed by endoscopes without any complication. The stents in the other dogs have been found in the correct position without serious macroscopical oesophageal injury.

Examination of the oesophagus wall

Macroscopic examination of the oesophagus in the group stent-1 showed gentle wall thickening and a touch of inflammation at the sites where the free metallic wire ends clung into the mucosa, whereas the mucosa underlying the PTFE membrane covering was smooth without signs of inflammatory or scarring. Wall thickening was observed alongside the stent in the group stent-2 without any sign of inflammation.

Microscopic examination of the distal oesophagus was performed in all cases. At dogs from group stent-1 without stent migration, injuries were limited to the region of the metal skirts at the two flared ends of the stent. Examination of the areas in contact with the covered middle part of the stent showed focal erosion of the mucosa exempt from inflammatory reactions (Fig. 2). Oesophagus samples from the group stent-2 with correct stent position, showed more explicit focal erosion of the oesophagus wall alongside where the stents were situated.

The microscopic investigation of the oesophagus in the other specimens (2 dogs) with alternating stent position has found normal mucosa and did not show reactive or dysplastic changes.

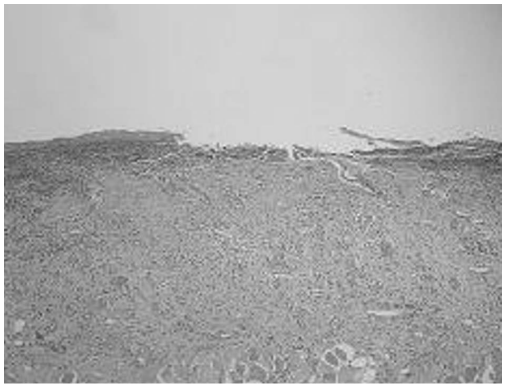

Fig. 2 Focal erosion of the mucosa in the group stent-1. Haematoxylin-eosin

StO_2 measurements

Figure 3 demonstrates alterations in tissue oxygen saturation values. Results are expressed as mean values ±s.d. StO_2 data were showed significant ($p<0.05$) decrease in StO_2 data in the group stent-2 in correlation with in the group stent-1 both examined oesophagus segment.

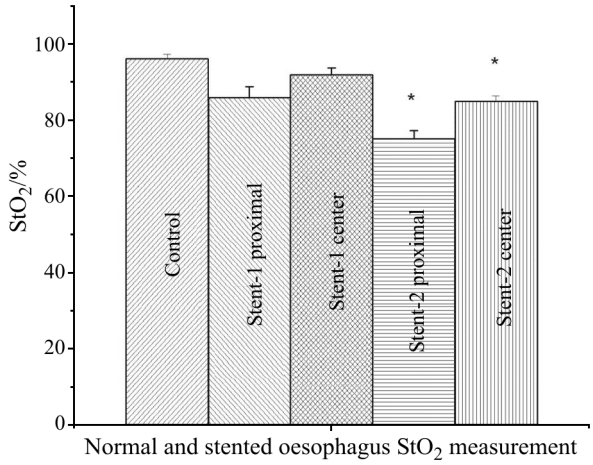

Fig. 3 The StO_2 data in the normal, and two different stented oesophagus. Significant decrease was seen in the group stent-2 in contrast to the group stent-1 both examined oesophagus segment

DSC measurements

According to the denaturing experiments the surgical interventions result in a significant alteration both in the temperature-course of DSC scans (Figs 4–6) as well as in their thermal parameters (Table 1) compared to the healthy control. Using the stent-1 the final results are closer to the healthy control, but its all sample exhibit more stable thermal data (greater calorimetric enthalpy and higher melting temperature) as the control or stent-2. In case of stent-2 all the total calorimetric enthalpy data are smaller than that of the same control or stent-1 parameters (we did not sepa-

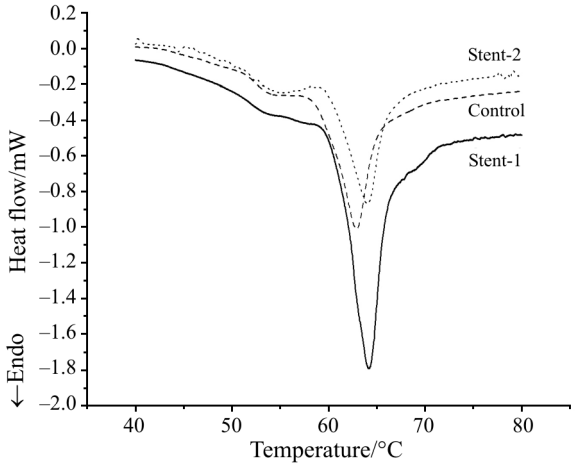

Fig. 4 Thermal denaturation of healthy and stented (stent-1, stent-2) dog whole oesophagus wall

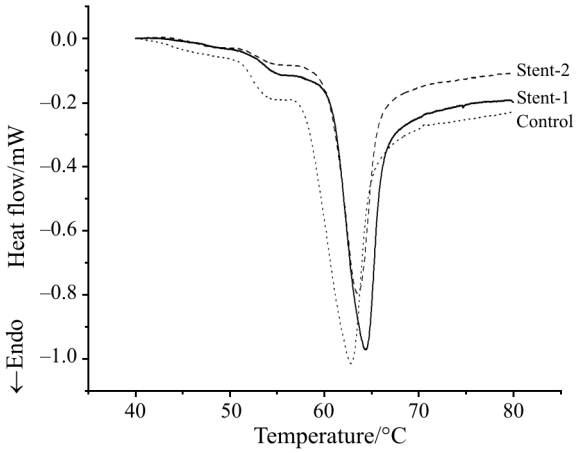

Fig. 5 Thermal denaturation of healthy and stented (stent-1, stent-2) dog oesophagus mucosa

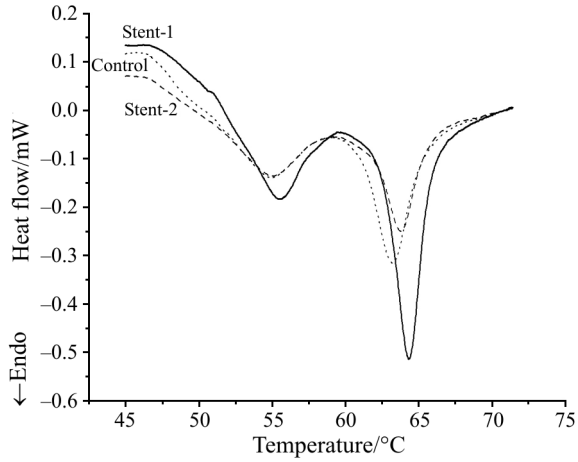

Fig. 6 Thermal denaturation of healthy and stented (stent-1, stent-2) dog oesophagus muscle fragment

Table 1 Thermal parameters of denaturation of oesophagus samples. Data are expressed in mean ±s.d. (in each case of four samples), the calorimetric enthalpy is normalised to unit sample mass

	Stent-1			Stent-2			Control		
	ΔH/J g^{-1}	T_{m1}/°C	T_{m2}/°C	ΔH/J g^{-1}	T_{m1}/°C	T_{m2}/°C	ΔH/J g^{-1}	T_{m1}/°C	T_{m2}/°C
total sample	2±0.1	54.2±0.3	64.1±0.3	1.34±0.07	55.2±0.3	64±0.3	1.6±0.08	54.9±0.3	62.9±0.3
mucosa	0.9±0.05	55.6±0.3	64.3±0.3	1.15±0.06	55.6±0.3	63.6±0.3	1.2±0.06	54.8±0.3	62.8±0.3
muscle	1.13±0.06	55.5±0.3	64.3±0.3	0.97±0.05	55.1±0.3	63.8±0.3	0.95±0.07	54.9±0.3	63.2±0.3

rated the two melting processes because their structural background is yet not known). These findings clearly demonstrate that despite of the significant alterations (increase of local pressure and stress) caused by the surgical intervention, stent-1 seems to be a good choice to improve the general state of patients in gastrointestinal bleeding.

Discussion

Massive bleeding from oesophageal varices is the major cause of death in patients with portal hypertension. To decrease the amount of blood loss is very important, and immediate management is required. In the acute phase of bleeding, 25% of the patients presenting with variceal haemorrhage continue to bleed, despite the acute drug or endoscopes therapeutic treatment [3]. These patients require further immediate intervention, usually a Sengstaken–Blakemore tube. The technique is successful in about 85% of cases, but the risk of recurrent haemorrhage following deflation is up to 50%. The device is uncomfortable for the patient and carries a 14% risk of serious complications, with 5% mortality; include aspiration pneumonia, oesophageal rupture and mucosal ulceration. Complications appear to be more common when balloon are placed by inexperienced personnel [2, 10, 11].

Placement of conventional oesophageal endoprosthesis and stents for palliation of obstructive oesophageal carcinoma is safe and well established [4, 5]. In 1957 Vossschulte described the first surgical implantation of metal cylinder in the distal oesophagus in the case of recurrent varix bleeding [12]. This method was not used again later on. In the beginning of the eighties palliative therapy of malignant strictures in the oesophagus was revolutionized by the use of SEMS [13]. Nowadays, the stent placement in the gastrointestinal tract increases fast, due to the better clinical conditions of the patient after the insertion, and to the easier feasibility of this method. Our aim was to evaluate a new method for decrease or stop the blood loss to stabilize the haemodynamic parameters in the acute phase of varix bleeding as well as to preserve the patient general wellness during the treatment.

This experiment showed from mechanical, histological and micro-circulation points of view that the new self-expandable SX-ELLA-Danis stent is a safe and suitable procedure without deterioration of the oesophageal wall when the stent size is comparable with the oesophagus dimension of an experimental animal. According to the DSC results the stent treatment has improved the thermal stability of the whole oesophagus as well as its main components (mucosa and muscle). It is very probably that beyond the urgent solution of acute problem of oesophageal varices the stent helps in the regeneration of the oesophagus (improved thermal parameters are the sign of stiffer structure) and this way makes the next coming interventions more successful.

Safety and efficiency in the experimental model had encouraged us to apply this method successfully on fifteen patients with bleeding oesophagus varices. The long term goal is to show that expandable metal stent placement could be an effective way of decreasing or stabilising the acute bleeding from ruptured oesophagus varices in cirrhotic patients until the effective therapeutic method (endoscopic band ligation, TIPS, etc.) would be introduced.

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