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The EsoCap-system – An innovative platform to drug targeting in the esophagus

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ABSTRACT

For the therapy of esophageal diseases such as eosinophilic esophagitis, there are no possibilities of local targeted therapy. This publication describes a novel, innovative drug delivery concept, that enables a targeted, long-lasting administration of drug substances to the esophageal mucosa. In addition to a comprehensive in-vitro characterization of the dosage form, this work includes a proof-of-concept study with healthy volunteers, which shows the functionality and acceptance of this novel drug delivery concept. This novel drug delivery technology enables for the first time a targeted, local and long-lasting therapy of the esophagus.

1. Introduction

Eosinophilic esophagitis (EoE) is a chronic, local immune-mediated esophageal disease, characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation [1,2]. While the first case was described in the late 1970s and EoE was found to be a rare disease, the latest data has since shown an increase in incidence and prevalence [3–7]. EoE is considered to be one of the most common causes of chronic esophagitis, after gastro-esophageal reflux disease (GERD), and is the leading cause of dysphagia and food impaction in children and young adults. EoE is characterized by a variety of symptoms, ranging from difficulty swallowing, food refusal, regurgitation and emesis, to dysphagia and food impactions [8].

Diagnosis of EoE is based on symptoms caused by esophageal dysfunction, endoscopic findings, or histologic features. It is challenging, however, as no single symptom in itself leads to diagnosis of EoE [9]. In clinical practice, EoE is suspected when a patient presents with symptoms of dysphagia, food impaction, or in children with feeding intolerance, abdominal pain, or vomiting. In adults and adolescents, dysphagia affects between 25% and 100% of EoE patients. Today EoE is recognized as the underlying cause in many cases of food impaction, requiring emergent evaluation and bolus clearance. In rare cases of food impaction, esophageal rupture may even occur [2,10]. Some patients also tend to suffer from retrosternal pain that is independent of the swallowing act [5,11]). The presentation is less specific in infants and toddlers. Symptoms mimicking GERD, such as dysphagia and food impaction, feeding intolerance, failure to thrive, abdominal pain, chest pain, nausea, vomiting and regurgitation are more frequent. Nausea, vomiting and stomach pain are also observed in school-age children.

Several published clinical interventional studies and case series have studied the effect of drugs on eosinophilic esophagitis. Most common is the use of topical corticosteroids, which are the treatment of choice for EoE. As an example, Bergquist et al. reported a clinical study with a suspension containing mometasone furoate [12]. Furthermore, different vehicles and dosage forms, such as aqueous suspensions, powder inhaler, syrups, maple syrup, agave nectar and other food products for local treatment of the esophageal mucosa have been reported [13–18]. Despite many corticoids having been used for the treatment of EoE, the only corticosteroid currently approved for treatment of EoE is budesonide (Jorveza), formulated as an orodispersible tablet [19]. A randomized trial showed the importance of maximizing esophageal contact time for an improved histological response rate [20]. The EoE therapy is most effective as a combination of long mucosal contact time with maximum deposition of topical steroids. This has been confirmed in clinical practice, supported by various publications [2,9,11,13]. It was observed that histological improvement in EoE patients was directly related to higher corticosteroid mucosal contact time, which can only be achieved in the treatment of EoE with appropriate drug delivery methods [11,13,18–20]. Nevertheless, there is a

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huge need for better pharmacological therapy for the esophagus [13], due to possible side effects in the throat caused by corticosteroids and short drug contact time with the esophageal mucosa.

The aim of this work is to develop a novel dosage form for local esophageal therapy and the corresponding application tools, which have been manufactured using innovative production technologies, such as 3D printing, and to investigate the fundamental applicability of a novel dosage form, called EsoCap. Various in-vitro tests and a proof-of-principle study in healthy volunteers were therefore performed. Within the scope of the study presented here, the novel dosage form was prepared for in-vitro characterization with a model drug. For the proof-of-principle study, a film that is visible using magnetic resonance imaging (MRI) was developed to visualize application of the film within the esophagus.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol 18–88 (PVA) was kindly gifted by Merck (Germany). Glycerol as plasticizer was purchased from Caelo (Germany). Demineralised water was used as a solvent. Fluorescein sodium as model substance was purchased from Sigma-Aldrich Chemie (Germany). Fluorescein-Sodium was used as a highly water soluble model substance to characterize the dissolution behavior of the film and the erosion of the polymer. Other, perhaps more relevant drugs could give less information about the dissolution behavior due to a poorer solubility. Food grade polylactic filament (Formfutura EasyPLA, Netherlands) was used for 3D printing of the drinking cup and applicator prototypes. Hibiscus tea was purchased from Spinnrad (Germany). A food-grade polyester string was purchased from Westmark (Germany). Calcium dihydrogen phosphate (JRS Pharma, Germany), magnesium stearate (Sigma-Aldrich Chemie, Germany), croscarmellose sodium (JRS Pharma, Germany) and black iron oxide (Caelo, Germany) were used for preparation of the sinker.

3. Methods

3.1. Film preparation

3.1.1. API containing film

The solvent-casting evaporation method was used for film production. The 22 cm long and 4 mm wide polymer film consists of polyvinyl alcohol (PVA) type 18–88, glycerol and purified water as the solvent during production. Fluorescein sodium (FS) was used as a model drug. The solvents were mixed in a laboratory glass bottle and heated up to 90 °C in a water bath, under constant stirring, for two hours. The mass was continuously stirred for another 60 min at 90 °C after addition of FS. The following production steps were carried out under exclusion of light for stability reasons. After three hours of heating, the mass is then stirred overnight, until cold (50 rpm), in order to prepare a bubble-free batch. The mass was drawn out on a liner process at a gap height of 1000 µm and dried at room temperature. The films were cut into elongated pieces of 0.4 cm × 22 cm and stored in airtight aluminium sachets until analytical investigations were performed.

3.1.2. MRI contrasting film

An aqueous hibiscus concentrate was used as a solvent for production of the MRI contrasting film. Aqueous hibiscus tea is known to be used as an oral negative contrast agent for MRI [21]. The 22 cm long and 0.4 mm wide polymer film (mass approx. 175 mg) consists of polyvinyl alcohol (PVA) type 18–88, glycerol and aqueous hibiscus concentrate, which is used as a solvent for the PVA during production. The hibiscus concentrate was prepared by extraction of 50 g ground hibiscus tea with 200 mL boiling water for approx. 12 h, followed by centrifugation. The solvents were mixed in a laboratory glass bottle and heated up to 90 °C in a water bath, under constant stirring. After six hours the mass is then stirred until cold (50 rpm). A maximum of 24 h before in-vivo MRI testing, the mass is drawn out on a liner process at a gap height of 1000 µm and dried at room temperature (relative humidity about 30%). The films were cut into elongated pieces of 0.4 cm × 22 cm and stored in airtight aluminium sachets until in-vivo testing was performed.

3.1.3. Assembly of EsoCap

The EsoCap for esophageal drug delivery consists of several parts: the rolled-up film, the slitted capsule, the sinker device and the retainer.

A hard gelatin capsule size 00 was used as a shell around the rolled-up film. For application, a slot was inserted using a mini electric circular blade.

The bottom of the hard capsule contains a compressed sinker as weight-reducing buoyancy. This sinker device was produced from a powder mixture (calcium dihydrogen phosphate 93.6%, sodium croscarmellose 5.0%, magnesium stearate 1.0%, iron oxide 0.4%) using a single punch tablet press (KP2, VEB Kombinat NAGEMA); diameter: 7.00 mm, weight: 515 mg). The upper end of the polymer film, which protrudes through the slit in the hard capsule, is fixed to a retainer thread made of food-grade polyester (Fig. 1).

The thread is in turn connected to an application beaker filled with water. For successful and uniform application of the EsoCap, the dosage form is located in a 3D-printed applicator, which is in turn connected to a drinking cup. A schematic representation is shown in Fig. 2. The applicator and the drinking cup are made of food-grade PLA filament, using a fused deposition modeling 3D printer (Ultimaker 3, Ultimaker BV, Netherlands).

The retainer is necessary to prevent early unrolling of the film in the mouth and throat during application. The retainer consists of a food-safe yarn that may be brought into contact with the human mucous membrane.

3.2. Characterization of the film

The film thickness was measured by a mechanical thickness dial gauge (0.01 mm capacity, Kaefer Messuhrenfabrik GmbH & Co.KG, Germany). Furthermore, ten samples (size 0.4 cm × 10 cm) were randomly selected, weighed and dissolved in 100 mL phosphate buffer pH 7.4 USP. After complete dissolution, samples were measured by UV/VIS spectroscopy, with a fiber-optics based system (Cary® 60, Agilent Technologies, USA, slit width 10 mm, wavelength 491 nm, baseline correction against 550 nm). The films passed the content uniformity test if they met the requirements of Chapter 2.9.6 Content Uniformity, of the European Pharmacopoeia 10.0 (Ph. Eur. 10.0).

For the paddle and glass disk (PGD) method, the DT 70 dissolution apparatus (Pharmatest, Germany) was equipped with paddles

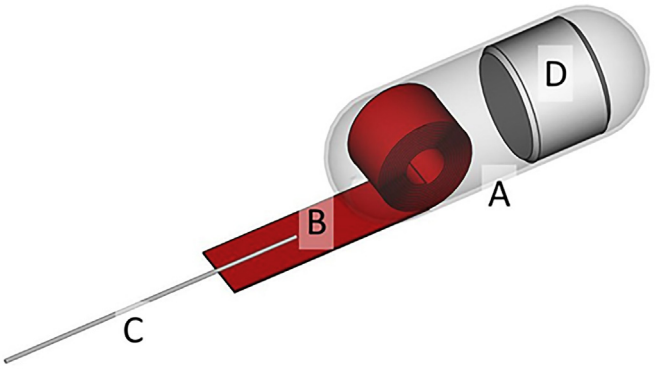

Fig. 1. Schematic drawing of the EsoCap (A: hard gelatin capsule; B: rolled-up, mucoadhesive film; C: retainer thread; D: compressed sinker).

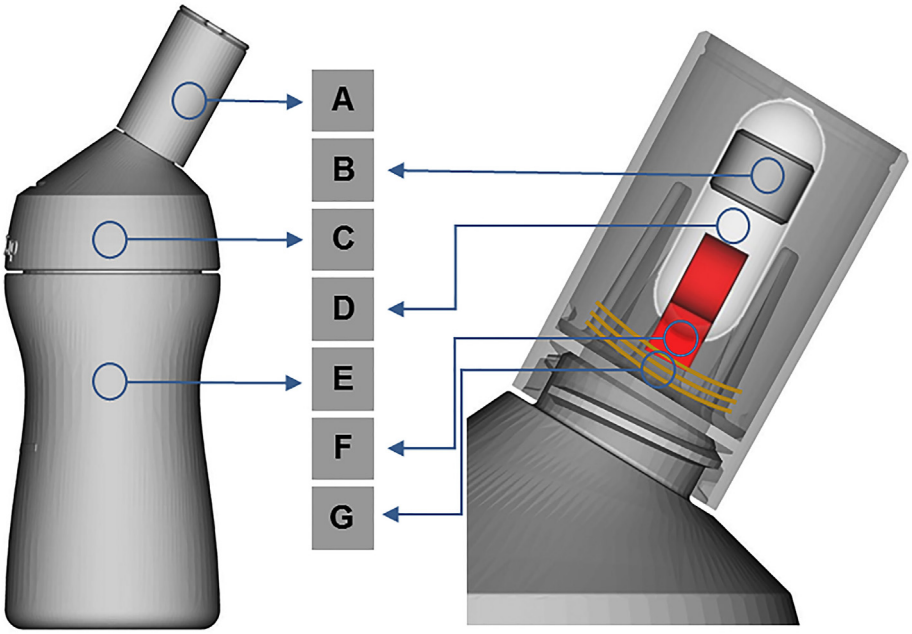

Fig. 2. Left – schematic drawing of the application device; right – close-up view of the applicator with EsoCap (A: applicator; B: compressed sinker; C: lid; D: hard gelatin capsule with slit; E: drinking cup; F: rolled-up, mucoadhesive film; G: retainer thread).

according to USP apparatus 2 and operated at a rotational speed of 50 rpm. Prior to the dissolution test, the films were attached by adhesive tape (type 64,620, Tesa, Norderstedt, Germany) on the drug-loaded side to glass disks 5 cm in diameter, which were subsequently placed centrally on the bottom of the dissolution vessels, below the paddle [22,23]. As dissolution media, 500 mL phosphate buffer pH 7.4 USP with a temperature of 37 °C was used. Drug release was measured via UV/VIS spectrometry, with a fiber-optics based system for on-line measurement (Cary® 60, Agilent Technologies, USA, slit width 10 mm, measuring interval 60 s, wavelength 491 nm). The dissolution testing was carried out in triplicate.

3.3. Proof-of-concept in vivo-study

3.3.1. Ethics

The prospective imaging study presented was conducted according to German MPG §23b, Good Clinical Practice and the Helsinki Declaration. All study-related documents were checked by the Ethics Committee at Greifswald Medical School and ethical approval was obtained (BB 170/18b). All subjects provided written informed consent for study procedures, including MRI, and were insured against any harm caused by study procedures and commuting accidents.

3.3.2. Subjects

The MRI studies were carried out in twelve healthy human volunteers: six male and six female subjects were recruited, with a mean age of 24.5 years (± 3.1 years) and a mean BMI of 23.0 kg/m^2 (± 2.3 kg/m^2). A table with the characteristics of the different study subjects can be found in the Supplementary Table 1. All subjects were ascertained to be in good health by means of their medical histories and physical examinations and checked for MRI exclusion criteria, such as metallic implants. The inclusion criteria were closely adapted to FDA and EMA guidelines for bioavailability and bioequivalence studies. All the subjects were non-smokers, had no history of gastrointestinal disorders or gastrointestinal surgery, no history of alcohol or drug abuse, abstained from alcohol for 48 h before study procedures and took no medication known to affect GI physiology. Female volunteers were checked for absence of gravidity by urine pregnancy test. During the study procedures, the participants were not allowed to drink or eat anything else but the water provided for intake of the EsoCap. The subjects received an appropriate expense allowance.

3.3.3. Study protocols

Reference images were taken before the EsoCap was administered. The EsoCap was placed in the applicator (as described before), before being taken by the test person. The applicator contained 100 mL local still drinking water for intake of the capsule. The EsoCap was applied in the upright position, in front of the tomograph, immediately after the reference images had been taken. The retainer thread attached to the applicator ensured that the polymer film rolled off in the esophagus during swallowing. The intake procedure was not allowed to exceed 2 min. After completion of the swallowing process (as subjectively perceived by the test person) and at least 2 min' wait, the thread could be swallowed or pulled out after successful intake. Fig. 3 Shows intake of the EsoCap. Images were taken at 2 min, 5 min, 10 min and 15 min after intake. At the end of the imaging process, the subject was given a standardized questionnaire to assess swallowability and negative sensations during intake. Swallowability and choke impulse were evaluated using a visual analogue scale, with 0 as the best result (e.g. swallowability like water, or no choke impulse) and 100 as the worst result (e.g. impossible to swallow, or vomiting). This swallowability score was based on the visual analogue scale as a semi-quantitative method for the subjective measurement of the strength of sensation like pain. This kind of questionnaire can be used to determinate the swallowability [24–27]. Water was provided ad libitum after completion of the measurements. Each subject had to take the EsoCap three times, on different study days, with no defined wash-out period.

3.4. Magnetic resonance imaging

Magnetic resonance images were taken using a commercially available Siemens MAGNETOM Aera (Siemens Healthcare, Erlangen, Germany) tomograph, with a magnetic field strength of 1.5 Tesla, at the Institute of Diagnostic Radiology and Neuroradiology (Greifswald). All measurements were carried out in the supine position. Strongly T1-weighted VIBE sequences were used in the sagittal orientation for visualization of the polymer film in the esophagus. If necessary, transversal slices were also obtained to ensure visibility of the capsule or

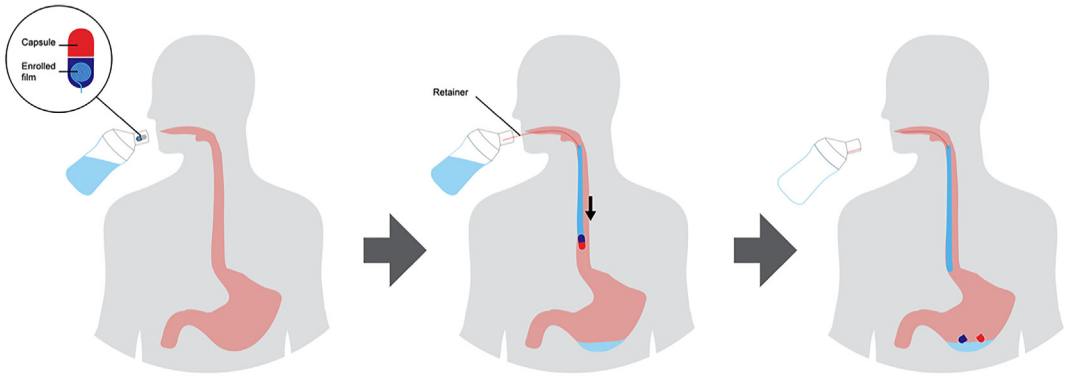

Fig. 3. Diagram of EsoCap application.

polymer film. The sequences had a repetition time of 3 ms, an echo time of 1.4 ms, a slice thickness of 2.5 mm, no interslice gap and a flip angle of 30°. The number of slices, phase oversampling and thus acquisition time were adapted according to individual anatomy. All acquisitions were performed within a single intake of breath, to reduce motion artifacts.

3.5. Image analysis

The MR image sets obtained were evaluated using Horos v2.2.0 freeware (The Horos Project). In addition to detecting the unrolled polymer film in the esophagus, their unrolled length was measured in vivo, as well as the duration of visibility at the site of application. All measurements were performed by three independent observers with experience in evaluation of MRI data. Means were calculated if differences between observations were less than 10% of measured length. If greater differences occurred, the results were discussed until a consensus was reached. 3D reconstructions and signal-intensity projections were used for visualization.

4. Results

4.1. Characterization of the film

The films had a smooth, homogenous and air-bubble free surface, as is demonstrated in Fig. 4. The MRI contrasting film containing hibiscus tea had an average thickness of 222 µm ± 2%. Compared to this, the film containing the model drug fluorescein sodium showed decreased thicknesses (average thickness: 120 µm ± 2%). The average content of ten samples from the polymer film containing fluorescein sodium showed a content of 0.019 mg/mg. Furthermore, all content values ranged between 90% and 110% of theoretical content and no sample was between 75% and 125% of the theoretical content. Accordingly, all investigated films passed the content uniformity test.

The dissolution profile is shown in Fig. 5. An estimated 80% of the drug is released after 25 min. The release reaches a plateau after about 60 min.

4.2. Proof-of-concept in vivo study

The in-vivo study showed that all 12 subjects were able to take the EsoCap successfully on three consecutive study days. No negative effects, such as nausea and vomiting, were observed during application. A generally good swallowability and acceptance were documented by means of the questionnaire filled out after application. This is expressed in a swallowability score of 17 ± 20% (n = 36). Furthermore, it turned out that the 3D printed drinking cup with applicator is suitable for successful application of the EsoCap. In addition, successful placement of contrast-enhanced polymer film on esophageal mucosa was observed in all cases (n = 36). Moreover, hyperintense areas in the esophagus were visible after 25 of 36 administrations, until the end of the MRI measurements (15 min after intake). Fig. 6 shows MRI images taken before and after application of the EsoCap system. The contrasted polymer film is clearly visible on the MRI image after intake. The arrows in the illustration mark the beginning and the end of the EsoCap film.

Furthermore, Fig. 7 shows transverse images of the highly visible, contrasted polymer film in the lower esophagus. It is also visible that there is no cavity between the polymer film and the collapsed esophagus. A video that shows the MR sequences can be found in the supplementary material.

Fig. 8 shows the results of the visible length determination.

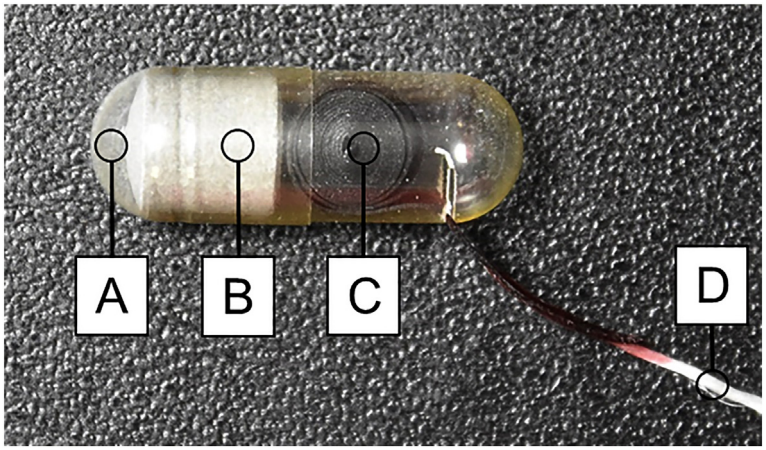

Fig. 4. Picture of the assembled EsoCap (A: hard gelatin capsule; B: compressed sinker, C: rolled up, mucoadhesive film; D: retainer thread).

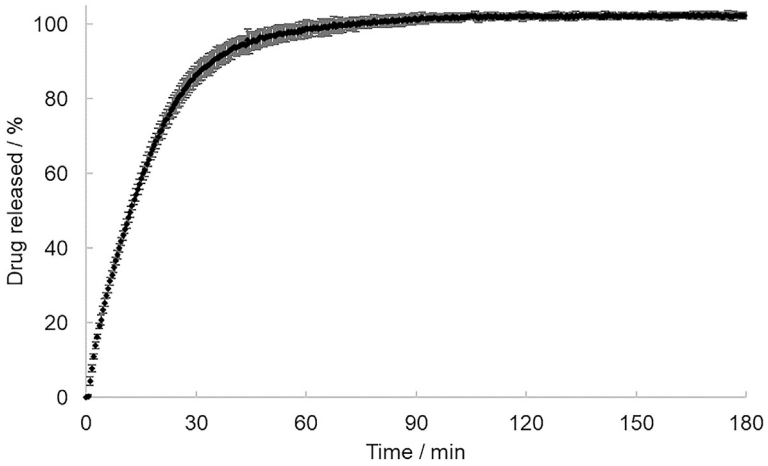

Fig. 5. Dissolution profile from fluorescein sodium film (mean of n = 3, ± SD).

Depending on the day of administration, a mean length of hyperintense area from polymer film between 9.6 ± 4.9 cm, 7.9 ± 2.8 cm and 6.8 ± 3.7 cm (n = 12 each) was detected.

5. Discussion

Investigation of dissolution in the conventional, but not biorelevant paddle apparatus, showed an active substance release, by dissolving the base polymer, of 80% after 25 min in 500 mL phosphate buffer pH 7.4. Compared to the relevant biorelevant parameters, such as a significantly lower existing fluid volume, it can be assumed that the time the active substance is in contact with the mucosa is significantly longer when applied in humans. A prolonged contact time is also associated with higher therapeutic success [20].

The proof-of-concept study in 12 healthy volunteers was carried out for investigation of principal functionality and acceptance of the EsoCap system in humans. A film containing hibiscus tea extract was therefore used as a contrasting agent. The study impressively showed that it was possible to administer EsoCap successfully 36 times. No side effects, such as nausea or vomiting, were observed despite the water-insoluble retainer combined with the novel application form.

Although this might seem surprising, the results fit well with experiences with a comparable system, the Cytosponge cell collection device. First described in 2009, this device consists of a sponge inside a capsule. The sponge is pulled back through the esophagus via an attached string after the capsule has been swallowed and has disintegrated [28,29]. This way, cells from esophageal mucosa can be scraped off and can be sampled without the need for endoscopic intervention. Several studies have proven the acceptability and safety of this method, as well as its clinical usefulness and diagnostic robustness [29–31].

A study of social media comments about the Cytosponge revealed mainly positive approaches to such an unusual device. The same might be expected for the EsoCap. The negative comments on the Cytosponge mainly addressed the conceivable risk of gagging and vomiting, which might be expected of the EsoCap, but which could be precluded in the present study. Our in-vivo study showed that only the string under tension from mouth to larynx is associated with uncomfortable sensations. A detailed explanation in the run-up to the study led to relaxation and successful application of the EsoCap in the volunteers. By giving the

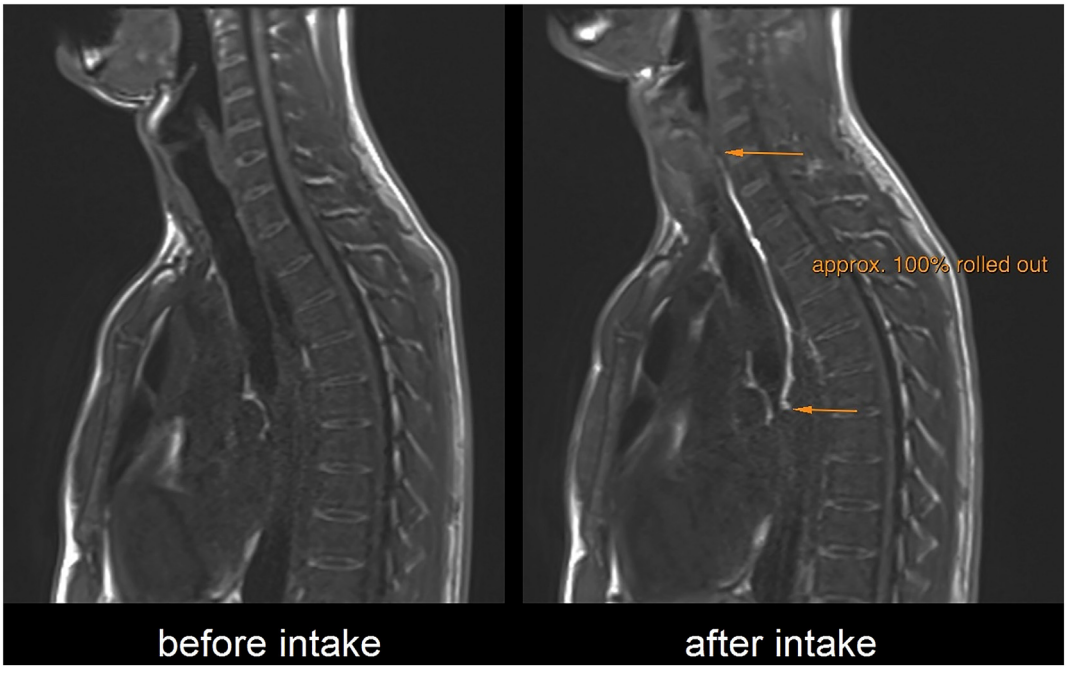

Fig. 6. T1-weighted sagittal magnetic resonance images before and after intake of the novel esophageal drug delivery system, with contrast-enhanced polymer film.

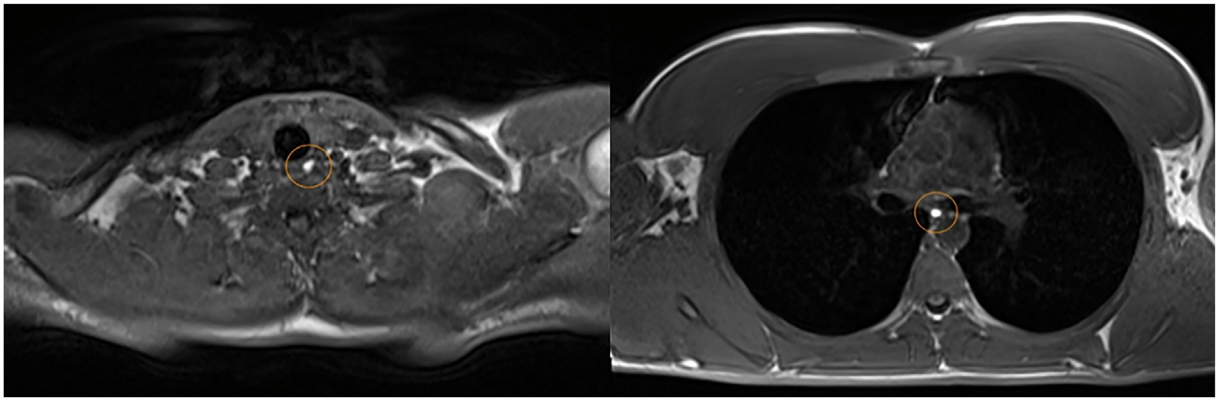

Fig. 7. T1-weighted transversal images with brightly visible polymer film in upper esophagus at the level of collarbone (left) and in lower esophagus near the heart (right).

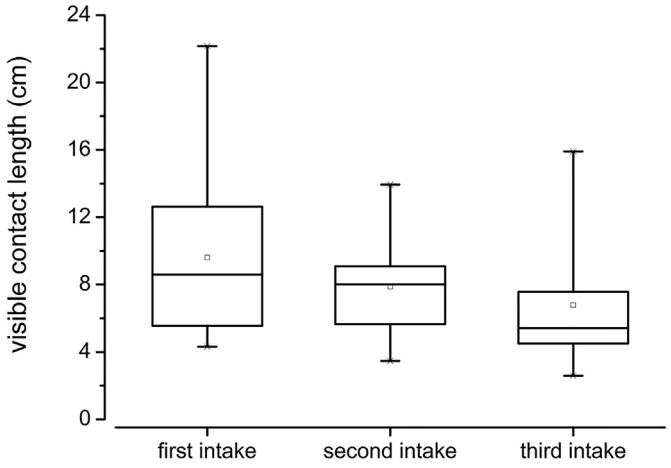

Fig. 8. Esophageal contact length of polymer film measured by MRI (whisker 5–95%, n = 12 each).

polymer film enough time to adhere to the mucosa and for swelling, the string could easily be extracted by the volunteers themselves after about three minutes, without noteworthy discomfort. In order to provide the patient with a better and even safer application experience, this retainer should in future be made of water-soluble or biodegradable polymers, which doesn't have to be removed after application. The volunteers did not describe the polymer film itself as foreign body inside the esophagus, which is comparable to the Cytosponge. In addition, this fits well with results from tracking studies with common hard capsules. It was shown that hard capsules are very likely to get stuck in the esophagus if not administered with enough water, but this was not felt by the subjects in the respective studies either [32–34].

Possibly the main and unique benefit of the EsoCap system for the treatment of local esophageal diseases, apart from its contact length, is the prolonged contact time on the esophageal mucosa. In the MRI study, the polymer film was visible for more than 15 min in 69% of all administrations. It is most likely that in some cases it would have been detectable even longer, but imaging was stopped according to the protocol. In cases where the film was not visible for the whole imaging time, this does not necessarily mean that it was not present anymore. The contrasting constituents of the film were probably washed out by salivary flow from the swollen polymer, due to use of very hydrophilic contrast substances from hibiscus tea. By comparison, previous studies investigated the esophageal clearance of highly viscous fluids used for EoE therapy. Hefner et al. showed a complete clearance of different viscous fluids after three minutes [17]. Since the residence time is an important factor for the success of local therapy of esophageal diseases, application of a mucoadhesive film by means of the EsoCap promises significant advantages.

6. Conclusion

In summary, it was possible to show that the novel EsoCap dosing system allows targeted, local therapy of esophageal diseases. An MRI study in healthy volunteers demonstrated the basic feasibility of the novel delivery concept. The EsoCap drug delivery system thus enables a targeted, long-lasting administration of drug substances to the esophageal mucosa, in the form of a mucoadhesive foil, for the first time. The foil contact time can be varied by using different polymers and by choosing the time of administration. Furthermore, using the EsoCap technology, a regional localization of drug application within certain sections of the esophagus, by sectional drug loading of the mucoadhesive film could be possible. The EsoCap system thus enables the possibility for a very variable and attractive platform for local therapy of diseases of the esophagus. Further in vivo studies with drug loaded EsoCap systems will be necessary to demonstrate the clinical benefit of this novel drug delivery technology.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jconrel.2020.08.011.

Data availability

The MRI data that support the findings of this study may be available on request from the corresponding author W.W., depending on requested information. The data are not publicly available due to them containing information that could compromise research participant privacy or consent according to German Data Protection Act. Explicit consent to deposit raw-sequencing data was not obtained from the subjects.

Data from in vitro characterization and results from MRI study are available on request from the corresponding author W.W.

Declaration of Competing Interest

A.R., M.G., C.R, J.K., N.H. and R.K. have no competing interests. W.W. is a co-founder and consultant of the EsoCap AG (Basel, Switzerland).

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