Advances in imaging and endoscopic therapy in Barrett’s esophagus
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Simultaneous use of endoscopic resection and radiofrequency ablation is not safe in an esophageal porcine model


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Part III: Experimental endoscopy: stenosis and endoscopic therapy in a porcine model

Abstract

Introduction: Radiofrequency ablation (RFA) is safe and effective for eradication of Barrett's esophagus after endoscopic resection (ER) of neoplasia. Widespread ER, however, is likely to induce stenosis, hampering subsequent circumferential RFA. A “single step” procedure with ER and circumferential RFA in the same session may avoid this problem. Two variants are possible: circumferential RFA of Barrett’s esophagus including the lesion followed by ER of the ablated lesion (“RFA→ER”); or ER of the lesion directly followed by circumferential RFA of remaining Barrett’s esophagus including the resection wound (“ER→RFA”). Aim: First, to evaluate perforation risk of “ER→RFA” using increasing RFA energies. Second, to compare stenosis rate after “ER→RFA” vs. “RFA→ER”. Methods: Experiment-1: 24 areas in 6 pigs underwent widespread ER directly followed by circumferential RFA with increasing energies (2x10, 2x12-6x12J/cm²) in the esophagus. Experiment-2: 8 pigs each had 4 treatment areas randomized: “ER→RFA”, RFA alone, ER alone, and “RFA→ER”. Results: No acute perforations occurred when ablating ER wounds. Two delayed perforations occurred: one in experiment-1, another in experiment-2 at the “ER→RFA” area. The remaining 7 pigs in experiment-2 showed stenosis in all “ER→RFA” and “RFA→ER” areas versus 5/7 RFA alone areas, and 0/7 ER alone areas. Conclusions: The “single step” variant “ER→RFA” is not safe in this porcine model and seems therefore not ethical to evaluate in humans at this stage. Given the high rate of stenosis after “RFA→ER” and RFA alone one might question the validity of the porcine model for this type of experiments.
Introduction

Radiofrequency ablation (RFA) has proved to be a safe and effective ablation technique for eradication of remaining Barrett’s epithelium after endoscopic resection (ER) of neoplasia.\(^1\)^

In this treatment protocol patients undergo ER of the neoplastic lesion, followed by circumferential RFA of the remaining Barrett’s epithelium at least six weeks later to allow the resection wound to heal. After the first circumferential RFA, subsequent RFA sessions are scheduled every 2-3 months until complete eradication of all visible Barrett’s epithelium is achieved. Most studies on the combination of ER and RFA, however, have limited the extent of the ER to 2 cm in length and including 50% of the circumference. By limiting the extent of the resected area, the risk of developing a severe stenosis was reduced.\(^5\)

Stenosis is not only a burden for the patient, but also makes circumferential RFA technically more difficult and comprises a higher risk of laceration or even perforation when performing RFA. As a consequence Barrett’s mucosa might be left untreated and subsequently new neoplastic lesions may occur that are inaccessible for endoscopic therapy. Although a limited ER is possible in most patients, a minority may harbor larger visible abnormalities (i.e. longer than 2 cm in length and/or involving >50% of the circumference) that need to be resected despite the high risk of stenosis. These patients may therefore benefit from a “single step” procedure in which ER and RFA are performed in the same treatment session. In this way problems associated with stenosis and circumferential RFA are overcome. Additional benefits of a “single step” procedure might consist of a reduction in number of treatment sessions which causes less burden to the patient and reduces costs.

Two variants for a “single step” procedure are possible, both having different drawbacks. One variant is ER of the neoplastic lesion directly followed by circumferential RFA of the remaining Barrett’s epithelium including the resection wound: “ER\(\rightarrow\)RFA”. The major drawback of this variant is the direct delivery of RFA at the resection wound. As a result, the muscularis propria may be seriously damaged resulting in severe stenosis or even acute perforation. The other variant of a “single step” procedure is circumferential RFA of the whole Barrett’s segment including the neoplastic lesion followed by ER of the ablated neoplastic lesion: “RFA\(\rightarrow\)ER”. In this sequence, delivering RFA on a resection wound is prevented. One major drawback of the “RFA\(\rightarrow\)ER” variant however is the increase in complexity of the subsequent ER. Because of edema and increased vulnerability of the mucosa after RFA the endoscopic view is impaired, hampering both the recognition of the electrocoagulation marks as well as the assessment of the quality of the submucosal lifting. Another drawback of the “RFA\(\rightarrow\)ER” variant is the histological evaluation of the resection specimen. As the neoplastic lesion is ablated immediately before ER, coagulation
artifacts may impair proper histological assessment of the lesion. In humans, “RFA→ER” has shown to be feasible and relatively safe, but the procedure was found to be technically demanding despite being performed by highly experienced endoscopists.6 “ER→RFA” is technically easier to perform but may result in complications. Therefore we aimed to explore the safety of “ER→RFA” and compare it to the safety of “RFA→ER” in a porcine model. The aim of the first experiment was to evaluate the perforation risk of “ER→RFA” using supratherapeutic RFA energy applications. The aim of the second experiment was to compare the number and severity of stenoses after “ER→RFA” and “RFA→ER” in a porcine model.

Methods

Animal handling
A total of 14 female pigs of 45-50 kg were included. Experiments were performed at the Animal Research Institute AMC (ARIA) after protocol approval by the Animal Ethical Committee at the Academic Medical Center in Amsterdam, Netherlands. Animal care was in accordance with European Union guidelines.

Endoscopic resection
ER was performed either with the multi-band mucosectomy technique (MBM, Duette™ kit, Cook Medical, Limerick, Ireland) or the cap technique with submucosal lifting (EMR Kit, Olympus Europe, Hamburg, Germany). Both techniques have been described previously in humans.7-10

Radiofrequency ablation system
The RFA system and endoscopic procedure have also been described previously in humans.1-4 Circumferential RFA was performed with the balloon based HALO³⁶⁰ system (BÂRRX Medical Inc., Sunnyvale, California, USA). The balloon based HALO³⁶⁰ system was chosen for several reasons. First, it is the easiest and less time consuming method of performing circumferential RFA and it is standard practice, especially in longer Barrett’s segments. Second, with focal devices it is still possible to ablate the resection wound itself at the edges, therefore still being necessary to evaluate the risks of RFA on the resection wound.

Experiment-1: short term safety of “ER→RFA”
In six pigs a total of 24 treatment areas were marked in the esophagus: in two immediately euthanized pigs 6 treatment areas and in four surviving pigs only 3 treatments areas for ethical
reasons. After sizing, ER (3 cm in length and 50% of circumference) with MBM of the targeted resection area within the treatment area was performed. Subsequently, each treatment area, including the resection wound, was ablated with the balloon indicated by the previous sizing. Treatment areas were ablated with different RFA energy applications without cleaning in between (Table 1). A top-down strategy was used to evaluate if supra-therapeutic RFA doses were safe (i.e. assessing safety margin). In addition, these different doses helped selecting the RFA dosis for experiment-2.

Two pigs were immediately euthanized after the experiment; two pigs were aimed to be euthanized after 1 day and two pigs after 3 days.

**Table 1.** RFA energy applications and aimed survival in experiment-1.

<table>
<thead>
<tr>
<th>Pig</th>
<th>No. “ER-RFA” treatment areas</th>
<th>RFA energy applications per treatment area from proximal to distal (J/cm²)</th>
<th>Aimed survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td>6</td>
<td>6x10; 5x12; 4x12; 3x12; 2x12; 2x10</td>
<td>0</td>
</tr>
<tr>
<td>Pig 2</td>
<td>6</td>
<td>2x10; 2x12; 3x12; 4x12; 5x12; 6x12</td>
<td>0</td>
</tr>
<tr>
<td>Pig 3</td>
<td>3</td>
<td>6x12; 5x12; 4x12</td>
<td>1</td>
</tr>
<tr>
<td>Pig 4</td>
<td>3</td>
<td>4x12; 5x12; 6x12</td>
<td>1</td>
</tr>
<tr>
<td>Pig 5</td>
<td>3</td>
<td>4x12; 2x12; 2x10</td>
<td>3</td>
</tr>
<tr>
<td>Pig 6</td>
<td>3</td>
<td>2x10; 2x12; 4x12</td>
<td>3</td>
</tr>
</tbody>
</table>

No number; “ER→RFA” single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; RFA radiofrequency ablation.

**Experiment-2: comparison of both “single step” procedures**

In each of the eight pigs 4 treatment areas were marked in the esophagus: “ER→RFA”, RFA alone, ER alone, and “RFA→ER”. Randomization of the treatment areas was performed prior to any treatment and resulted in: distal “ER→RFA”, mid-distal RFA, mid-proximal ER, proximal “RFA→ER”; or: distal “RFA→ER”, mid-distal RFA, mid-proximal ER, proximal “ER→RFA”.

After sizing, ER with the cap technique of the targeted resection area was performed within the “ER→RFA” and the ER alone treatment areas. Resection specimens were collected for histology. Subsequently, “ER→RFA”, RFA alone and “RFA→ER” treatment areas (including the ER wound of the “ER→RFA” area) were ablated with the balloon indicated by the previous sizing and using the 10J/cm²-clean-10J/cm² regimen. Next, ER with the cap technique of the targeted area within the “RFA→ER” was performed and resection specimens were collected. At the end of the procedure, all treatment areas were marked just proximal by placing two small tattoos (SPOT, GI Supply, Camp Hill PA, USA). All pigs were aimed to be euthanized 42 days after the experiment.
Follow-up

Pigs were euthanized with an intravenous overdose of pentobarbital after which the esophagus was harvested for histology. Surviving pigs received a semi liquid diet and were placed on a grid for 3 days in order to prevent sawdust perforating the esophageal wounds. After 3 days pigs progressively received a more solid diet. In case of regurgitation, i.e. stenosis, pigs were offered again a semi liquid diet which was supplemented with milk protein if additional caloric intake was necessary.

Histology

After harvesting the esophagus the treatment areas were identified by opening the esophagus in longitudinal direction. The treatment areas were cut out and stretched on paraffin with pins. In addition, in experiment-2 the mucosal circumference of each treatment area was measured with a ruler at the center of the treatment area and at the upper edge of the treatment area. ER specimens in experiment-2 were retrieved immediately after resection, pinned down on paraffin, and fixed in 10% formalin solution. After fixation specimens were processed and stained with haemotoxilin & eosin.

Histological evaluation was performed by a gastro-intestinal expert pathologist (MV). Specimens were evaluated for the presence and depth of inflammation, fibrosis and necrosis. The deepest layer with damage due to inflammation, fibrosis and/or necrosis was recorded.

Endpoints

Primary endpoint for experiment-1 was:

1) Number of acute and delayed perforations after “ER→RFA” using supratherapeutic RFA energy applications;

Secondary endpoint for experiment-1 was:

1) Depth of inflammation and/or necrosis in the esophageal wall at day 0, 1 and 3 on histology after “ER→RFA” using supratherapeutic RFA applications.

Primary endpoint for experiment-2 was:

1) Number and severity of stenosis after “ER→RFA”, “RFA→ER”, RFA and ER.

Secondary endpoints for experiment-2 were:

1) Depth of fibrosis in the esophageal wall on histology after “ER→RFA”, “RFA→ER”, RFA and ER.

2) Depth of necrosis on histology of resection specimens after “ER→RFA”, “RFA→ER”, RFA and ER;

3) Number of acute and delayed perforations after “ER→RFA”, “RFA→ER”, RFA and ER;
**Statistical analysis**
Statistical analysis was performed with the Statistical Software Package version 16.0.2 for windows (SPSS, Chicago, Illinois, USA). For descriptive statistics, mean with standard deviation was used for variables with a normal distribution. One-Way Anova was used to compare the multiple treatment regimens in experiment-2.

**Results**

**Experiment-1: short term safety of “ER→RFA”**

*Perforations*
No acute perforations occurred when ablating the ER wounds. During the MBM procedure, prior to RFA, acute perforation occurred in 2 of the 24 treatment areas; both perforations were located in the distal esophagus. One delayed perforation occurred at the 5x12J/cm² treatment area in one of the four pigs of the survival experiment.

*Depth of damage in esophageal wall*
Depth of damage on day 0, 1 and 3 are shown in Table 2. Although damage was present on day 0, no inflammation or necrosis was seen. At day 1 and 3 clear inflammation and necrosis was seen. Exact location of deepest inflammation and necrosis within the treatment areas was not possible to determine.
Table 2. Perforations and damage in experiment-1.

<table>
<thead>
<tr>
<th>Pig</th>
<th>Survival (Days)</th>
<th>RFA energy dose (J/cm²) of “ER → RFA” treatment areas</th>
<th>Acute perforation</th>
<th>Delayed perforation</th>
<th>Deepest layer with damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1</td>
<td>0</td>
<td>6x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5x12</td>
<td>No</td>
<td>-</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x12</td>
<td>No</td>
<td>-</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-† Yes at ER</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pig 2</td>
<td>0</td>
<td>2x10</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5x12</td>
<td>No</td>
<td>-</td>
<td>Submucosa</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6x12</td>
<td>No</td>
<td>-</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td>Pig 3</td>
<td>1</td>
<td>6x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td>Pig 4</td>
<td>1</td>
<td>4x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5x12</td>
<td>No</td>
<td>Yes</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td>Pig 5†</td>
<td>1</td>
<td>4x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x12</td>
<td>No</td>
<td>No</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x10 Yes at ER</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pig 6</td>
<td>3</td>
<td>2x10</td>
<td>No</td>
<td>No</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2x12</td>
<td>No</td>
<td>No</td>
<td>Muscularis propria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4x12</td>
<td>No</td>
<td>No</td>
<td>Transmural</td>
</tr>
</tbody>
</table>

RFA radiofrequency ablation; “ER → RFA” single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; ER endoscopic resection.

†: This area was not treated with 2x10J/cm² as a perforation occurred during the endoscopic resection.
‡: This pig aimed to survive 3 days was euthanized at day 1 due to a severe infection.

Experiment-2: comparison of both “single step” procedures

Perforations and stenosis

No acute perforations occurred. One delayed perforation occurred in one pig at the “ER → RFA” treatment area causing a severe infection that required premature euthanasia at day 8. The remaining 7 pigs developed severe stenosis causing regurgitation and impeding proper caloric intake, therefore being prematurely euthanized after a mean of 23 days (range 16-30 days) (Table 3). In these 7 pigs number and severity of stenosis was evaluated. All “ER → RFA” and “RFA → ER” showed severe stenosis at the treatment area. RFA alone treatment areas showed stenosis in 5/7 pigs. None of the ER alone treatment areas showed stenosis (Figure 1). The severity of the stenosis based on the ratio of the circumference of the mucosa at the center and the edge of the treatment area was not significantly different between “ER → RFA” and “RFA → ER” (Table 4).
Table 3. Treatment area location and survival in experiment-2.

<table>
<thead>
<tr>
<th>Type of treatment per treatment area from proximal to distal</th>
<th>Survival (days)</th>
<th>Reason for euthanasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1 “RFA→ER” / ER alone / RFA alone / “ER→RFA”</td>
<td>8</td>
<td>perforation</td>
</tr>
<tr>
<td>Pig 2 “ER→RFA” / ER alone / RFA alone / “RFA→ER”</td>
<td>23</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 3 “RFA→ER” / ER alone / RFA alone / “ER→RFA”</td>
<td>22</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 4 “ER→RFA” / ER alone / RFA alone / “RFA→ER”</td>
<td>25</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 5 “ER→RFA” / ER alone / RFA alone / “RFA→ER”</td>
<td>21</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 6 “ER→RFA” / ER alone / RFA alone / “RFA→ER”</td>
<td>16</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 7 “RFA→ER” / ER alone / RFA alone / “ER→RFA”</td>
<td>24</td>
<td>stenosis</td>
</tr>
<tr>
<td>Pig 8 “RFA→ER” / ER alone / RFA alone / “ER→RFA”</td>
<td>30</td>
<td>stenosis</td>
</tr>
</tbody>
</table>

“RFA→ER” single step procedure with first circumferential radiofrequency ablation followed by endoscopic resection; ER endoscopic resection; RFA radiofrequency ablation; “ER→RFA” single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation.

Table 4. Severity of stenosis for the different treatment regimens in experiment-2 according to mucosal circumference ratio.

<table>
<thead>
<tr>
<th>Mucosal circumference at treatment area</th>
<th>Center (cm)</th>
<th>Edge (cm)</th>
<th>Ratio center/edge</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>4,6 (±1,8)</td>
<td>6,3 (±1,5)</td>
<td>0,72 (±0,15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RFA</td>
<td>3,3 (±2,7)</td>
<td>5,8 (±1,5)</td>
<td>0,52 (±0,30)</td>
<td>0.013</td>
</tr>
<tr>
<td>“ER→RFA”</td>
<td>1,4 (±0,3)</td>
<td>5,6 (±1,5)</td>
<td>0,27 (±0,14)</td>
<td>0.154</td>
</tr>
<tr>
<td>“RFA→ER”</td>
<td>1,2 (±0,3)</td>
<td>6,6 (±1,5)</td>
<td>0,18 (±0,06)</td>
<td></td>
</tr>
</tbody>
</table>

ER endoscopic resection; RFA radiofrequency ablation; “ER→RFA” single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; “RFA→ER” single step procedure with first circumferential radiofrequency ablation followed by endoscopic resection.

*: p-values calculated with One-Way Anova

Depth of damage in esophageal wall

Inflammation and/or fibrosis was present in all treatment areas. It reached the muscularis propria in 63% (5/8) of the ER en RFA alone treatment areas and 75% (6/8) of both “single step” procedures. Transmural inflammation and/or fibrosis in the muscularis propria was seen in 1/8 ER alone treatment areas and in 3/8 “ER→RFA” treatment areas. Exact location of deepest inflammation and/or necrosis within the treatment areas was not possible to determine.

Depth of damage in resection specimens

All resection specimens of “RFA→ER” were damaged: epithelium and lamina propria were missing, and in one case the muscularis mucosae was damaged as well. In all resection specimens of “ER→RFA” and ER alone all layers were present except the muscularis propria.
Summary of both experiments

Table 5 summarizes all endoscopic and histological endpoints for both experiments. Acute perforations were only seen during experiment-1 when using MBM in the distal esophagus leading to the use of the cap technique in experiment-2. Delayed perforations were only seen with “ER\rightarrow RFA”. In this porcine model the majority of the areas treated with RFA alone or in combination with ER leaded to severe stenosis. Damage in the esophageal wall reached the muscularis propria in the majority of all treatment modalities. Transmural damage was only seen after ER or “ER\rightarrow RFA”. Damage to resection specimens reached at deepest the muscularis mucosae in one case after the “RFA\rightarrow ER”.

<table>
<thead>
<tr>
<th></th>
<th>ER alone</th>
<th>RFA alone</th>
<th>“ER\rightarrow RFA”</th>
<th>“RFA\rightarrow ER”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute perforation</td>
<td>5%†</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Delayed perforation</td>
<td>0%</td>
<td>0%</td>
<td>11%‡</td>
<td>0%</td>
</tr>
<tr>
<td>Severe stenosis</td>
<td>0%</td>
<td>71%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Depth of damage esophageal wall:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>muscularis propria</td>
<td>63%</td>
<td>63%</td>
<td>63%§</td>
<td>75%</td>
</tr>
<tr>
<td>transmural</td>
<td>13%</td>
<td>0%</td>
<td>33%¶</td>
<td>0%</td>
</tr>
<tr>
<td>Depth of damage resection specimen:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>submucosa</td>
<td>0%</td>
<td>NA</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>muscularis mucosae</td>
<td>0%</td>
<td>NA</td>
<td>0%</td>
<td>13%</td>
</tr>
</tbody>
</table>

ER endoscopic resection; RFA radiofrequency ablation; “ER\rightarrow RFA” single step procedure with first endoscopic resection followed by circumferential radiofrequency ablation; “RFA\rightarrow ER” single step procedure with first circumferential radiofrequency ablation followed by endoscopic resection; NA not applicable.

†: 2 perforations occurred with the multi-band mucosectomy technique, none with the cap technique
‡: one perforation occurred with 5x12 J/cm² ablation after 1 day, the other with 2x10 J/cm² after 8 days
§: 64% for ≤2x12 J/cm² and 63% for ≥3x12 J/cm²
¶: 21% for ≤2x12 J/cm² and 50% for ≥3x12 J/cm²
Discussion

This porcine model study suggests that the “single step” variant “ER→RFA”, i.e. ER directly followed by circumferential RFA including the resection wound, is not safe. Immediate ablation of the fresh resection wound did not cause any acute perforations, but two delayed perforations did occur several days after the procedure. These delayed perforations not only occurred in a pig treated with a supratherapeutic RFA energy dose (≥3x12J/cm²) in experiment-1, but also in a pig treated with a low therapeutic RFA energy dose (10J/cm²-clean-10J/cm²) in experiment-2. These delayed perforations probably reflect the depth of the damage caused by RFA on the resection wound. When evaluated immediately (day 0) after the procedure the damage seemed limited, however, much deeper damage caused by necrosis and inflammation became visible after 1 day. Clear transmural damage of the muscularis propria was seen in all supratherapeutic RFA energy applications in experiment-1 and in a considerable number of the low therapeutic RFA energy dose in experiment-2.

Number and severity of stenosis of both “single step” variants were compared in experiment-2. “ER→RFA” as well as “RFA→ER” resulted in severe stenosis in all cases. We tried to quantify the
severity of stenosis by calculating the ratio of the mucosal circumference at the center of the treatment area and that at the upper end of the treatment area. No significant differences in the severity of stenosis were seen between both “single step” procedures. As expected, ER alone did not result in stenosis of any treatment area as the resections were confined to 3cm length and 50% of the circumference.\textsuperscript{5} Surprisingly RFA alone resulted in stenosis in the majority of treatment areas. This in contrast to previous pioneering work on RFA in a porcine model showing no stenosis when using 9.7 or 10.6J/cm\textsuperscript{2} once, although stenosis was seen in up to 50% when using 11.5 or 13.3J/cm\textsuperscript{2} once.\textsuperscript{11} As we ablated twice with 10 J/cm\textsuperscript{2}, one might argue that this results in a higher dose, however, from studies in human esophagi it is known that ablating twice with 10 or 12J/cm\textsuperscript{2} does not result in significantly deeper damage than ablating once.\textsuperscript{12}

When comparing the depth of the damage of both “single step” variants, “ER\rightarrow RFA” resulted in transmural inflammation and fibrosis of the muscularis propria in 3/8 cases, while “RFA\rightarrow ER” did not result in transmural damage. Surprisingly, ER alone also resulted in transmural damage. The same observation has been made in dogs, data in humans are lacking.\textsuperscript{13} The damage of “ER\rightarrow RFA” might thus be partly explained by the effect of ER. Although RFA alone did not result in transmural damage, damage was as deep as the muscularis propria. The relatively deep damage seen after RFA in our experiments might be explained by several features. First, the mucosal layer of the porcine squamous esophagus is relatively thin compared to the human Barrett’s esophagus. Second, pigs were relative small and might therefore have had a thinner esophageal wall as well. Finally, the differences in wall architecture with a thick muscularis mucosae and almost absent submucosa in the distal esophagus and an almost absent muscularis mucosae with abundance of submucosal glands in the proximal esophagus of the porcine esophagus may also have resulted in the deep damage. All these factors may have contributed to the deep damage seen with RFA alone and might explain the high number of stenosis in our porcine model.

ER specimens of “RFA\rightarrow ER” showed superficial damaged due to the preceding RFA: epithelium and lamina propria were missing, and in one case even de muscularis mucosae was damaged. Nevertheless, the majority of specimens showed no deeper damage than the muscularis mucosae. In humans less deeper damage has been found in resection specimens after RFA which is probably a consequence of the thicker Barrett’s epithelium.\textsuperscript{6} As muscularis mucosae and submucosa remain present in ER specimens despite RFA, evaluating infiltration depth, differentiation grade, lymphangio invasion and therefore subsequent therapy are still adequately possible. The major limitation of this study is that the porcine esophagus and the human esophagus have some distinct differences. Although all layers present in a human esophagus are present in the porcine esophagus, the layers are much thinner. In addition, the muscularis mucosae is almost as thick as the muscularis propria in the distal part of the porcine esophagus, while the proximal
part has almost no muscularis mucosae and a very thick submucosa. The middle part of the porcine esophagus where both muscularis mucosae as well as submucosa are present, seems thus the most comparable to the human situation. Because of these differences in the esophageal wall architecture, our study results can not be completely extrapolated to the human Barrett’s situation.

Nevertheless our results suggest that “ER→RFA” should not be performed in humans. “RFA→ER” is, although less practical, likely to be safer. A series of 24 patients treated with the regimen has shown that this is a feasible approach in selected patients. The procedure is, however, technically more complicated. Although our study did not evaluate the increased complexity of the subsequent ER, subjectively this complexity was experienced during the “RFA→ER” procedures. In the future we might benefit more from measures that can prevent stenosis formation after extensive ER, allowing the conventional treatment sequence of ER followed by ablation after healing of the resection wound. Further research focusing on prevention of stenosis formation should be encouraged.

In conclusion, in this porcine model study, the “single step” procedure with ER immediately followed by circumferential ablation of the esophagus including the resection wound was associated with delayed perforation and transmural damage. Although this can not be completely extrapolated to humans, it seems not ethical to evaluate this “single step” variant further in humans. RFA of the esophagus including the neoplastic lesion immediately followed by ER of the ablated lesion, although cumbersome, seems safer and might have a place in the endoscopic treatment of patients with large neoplastic lesions in the Barrett’s esophagus.

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Part III: Experimental endoscopy: stenosis and endoscopic therapy in a porcine model

References


