Assessing the conditions for in vivo electrical virtual biopsies in Barrett’s oesophagus

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Abstract—It has previously been shown that it is possible to differentiate between squamous and columnar epithelia in rat and resected human tissues using an impedance probe to make in vitro measurements. This probe can be passed down an endoscope allowing measurements to be made in patients. However, the probe emerges parallel to the oesophageal wall, with little room to manoeuvre. The conditions of control required to give reliable readings have been investigated. The importance of pressure applied and the angle of approach to the oesophagus was assessed. Pressures in the range 26.6Pa to 46.3kPa and angles in the range 15–90 degrees were considered. In in vitro studies it was observed that it was possible to obtain consistent readings with pressures greater than 2.9kPa and with angles greater than 15 degrees between the probe and the oesophagus. These conditions can be achieved in vivo, and readings obtained from twelve patients are shown (45 readings on normal squamous, 34 on Barrett’s oesophagus and 22 on stomach). At low frequencies (9.6–153.2kHz), a Mann–Whitney test shows a significant difference (p<0.001) when comparing the means from squamous and columnar, and also when readings from Barrett’s and normal gastric epithelia are compared (p<0.001).

Keywords—Virtual biopsies, Barrett’s oesophagus, Bio-electrical impedance

1 Introduction

BARRETT’S OESEPHAGUS (BO) is a pre-cancerous condition in which the normal squamous epithelium of the oesophagus is replaced by columnar epithelium of the gastric or intestinal type (SPECHLER, 1994). It can have a patchy pattern or cover relatively large regions of the lower oesophagus. Sometimes, islands of squamous epithelium are found surrounded by columnar (Barrett’s) epithelium. The pathogenesis of BO is not very well known and for some time it was considered a congenital disease. It seems that due to gastro-oesophageal reflux, the squamous epithelium of the lower oesophagus is destroyed and replaced by columnar type. Once this change has taken place, the risk of developing adenocarcinoma is increased 30 to 40 fold (CLARK and DEMEESTER, 1995) compared with the general population.

The diagnosis of BO is made by means of biopsies taken trans-endoscopically (RIDDLEL, 1996). Patients with this condition are put in a surveillance programme which aims to diagnose pre-malignant changes in the early stages. The conventional approach is to take biopsies from each quadrant at 2 cm intervals along its length to examine each of the numerous specimens for dysplastic change. We have developed a probe suitable for taking trans-endoscopic electrical impedance readings (‘virtual biopsy’, GONZÁLEZ-CORREA et al., 1999) in the oesophagus and stomach, with which we expect to investigate the possibility of limiting real biopsies to just the few areas of abnormality identified by electric impedance. We have already shown (GONZÁLEZ-CORREA et al., 1999) that we can differentiate between squamous and columnar epithelia. This opens up the possibility for diagnosis of BO by this means. A step further in our investigation is to try to characterise the different stages of columnar epithelium from metaplasia to cancer (simple metaplasia, inflammation, dysplasia and malignancy). In this article we show the first in vivo electrical impedance readings made in twelve patients with BO and the steps that were necessary before taking them.

There were two main questions to be answered before trying any measurement in patients: (1) How much pressure do we need to apply to the probe to get a good contact between the electrodes and the tissue? (2) What is the minimum angle of approach between the probe and the tissue with which we can obtain acceptable readings?

2 Methods

The equipment used for the experiments was the same as that previously described (GONZÁLEZ-CORREA et al., 1999). To answer the first question mentioned in the introduction, we
carried out the following experiment using tissue taken from six rats: each rat was sacrificed under anaesthetized and the stomach was resected, extended on a small piece of cork (7.0 x 11.5 x 1.5 cm) and fixed with pins. This preparation was placed on an electronic balance. Twelve different pressures were applied, from about 26.6 Pa to about 46.3 kPa, each value being about twice the previous one. After each reading and before taking the next one the preparation was released to let the tissue recover its initial state. Once all 12 readings were completed at a specific point, another point was chosen and the same procedure was repeated. Taking the diameter of the probe tip as 3.32 mm, the pressures were calculated as the reading in grams multiplied by a constant (1132 Pa g⁻¹) to convert grams into Pa. The probe was fixed to a micromanipulator and pushed downwards until the balance gave a reading close to the desired one. Because tissue tends to redistribute, it was not possible to obtain completely steady readings on the balance and the pressure had to be adjusted several times before taking the electrical reading. We also carried out an experiment in which pressure was applied to the tissue until a small depression appeared under the tip of the probe. This was done ten times using different samples and when the depression was visible, the pressures were in the range 11.3 to 33.9 kPa.

To answer the second question formulated in the introduction, we used resected tissue from three patients with oesophageal adenocarcinoma, considering that the rat tissue was not suitable for this purpose as it is much thinner than the human tissue. In this case the tissue was extended on a table and readings were taken at different points, trying angles of approach (between the probe and the horizontal plane) of 90, 75, 60, 45, 30 and 15 degrees, and then back again (on the same point) from 15 to 90 degrees in steps of 15 degrees.

As tissue impedance always decreases with frequency, we have used this as one criterion in assessing data quality. Therefore, if we plot resistivity readings on the y-axis and frequencies on the x-axis (from the lowest at left to the highest at right) all curves are expected to go down from left to right. If any curve goes up anywhere, then this is evidence of a technical problem and the data has to be rejected. The highest readings are expected at the lowest frequency. Data in which the low frequency readings either are very close to the saturation point of the amplifiers (more than 9.5 volts at any gain) or are under 0.5 volts, where the response of the measurement system is non-linear, are also rejected.

Measurements on the oesophageal wall of twelve patients were carried out after obtaining approval of the Local Research Ethics Committee of the Rotherham General Hospital NHS Trust. A letter of consent were signed by all patients. Of all these measurements, only those from one patient did not have histopathological confirmation.

3 Results

In Fig. 1 we can see the curves obtained from squamous tissue when applying the twelve different pressures. Pressure increases from the first box (with about 22.6 Pa) to the last one (with about 46.3 kPa). Fig. 2 shows similar curves to those of Fig. 1, but taken on columnar tissue.

In rat squamous tissue, reliable readings were obtained from pressures of about 2.9 kPa and above as can be seen in Fig. 1. In rat columnar tissue a slightly lower pressure was needed to obtain reliable readings as seen in Fig. 2. A pressure of at least 1.41 kPa gave reliable results. Measurements of the pressure required to cause a significant depression of the tissue show that, at this point, we are well above the minimum pressure required to obtain reliable readings.

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Fig. 1 Electric impedance readings (n = 14) at different pressures on normal gastric squamous tissue using the foresomach of six rats. The y-axis is calibrated in terms of resistivity in Ωm and the x-axis is frequency (kHz) on a logarithmic scale.

Fig. 2 Electric impedance readings (n = 22) at different pressures on normal columnar tissue using the glandular stomach of six rats. Axes are the same as in Fig. 1.