Liver electrolysis: pH can reliably monitor the extent of hepatic ablation in pigs

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ABSTRACT
Electrolysis is a method of tissue ablation that creates chemical species and a pH gradient in response to direct current. Initial studies of electrolysis in animal models and humans have shown that it is a safe, predictable and effective process for destroying normal and tumour-bearing liver in a linear, dose-dependent manner. Presently, the amount of current that is applied (in coulombs) has to be calculated using historical data, with inherent inaccuracy. The present study tested whether pH could be used as a real-time monitor in order to predict more accurately the extent of necrosis. A total of 70 electrolytic lesions were created in 14 pigs, with pH monitoring of the lesion edge. The normal range of pH values was 6.5–8.7. A pH of less than 6 (at the anode) or more than 9 (at the cathode) reflected total cellular necrosis. When a pH value was recorded between 6.0 and 6.5 at the anode or between 8.7 and 9.0 at the cathode, the presence of necrosis was variable. In conclusion, during electrolytic ablation, pH measurement can monitor the extent of the induced necrosis.

INTRODUCTION
Patients with untreated colorectal liver metastases have a median survival time of less than 1 year [1–4]. Surgical resection offers the only real hope of cure, with 5-year survival of around 30–40% [1,3–6]. Resection, however, is only possible in about 20% of patients, who have the disease in favourable anatomical locations [1,3,5–7]. In recent years ablative techniques have attracted much interest, as they may allow a less morbid procedure, may palliate or may allow staged resection [3,4,6–9]. Up to 40% of colorectal metastases are confined to the liver [10,11]. In these patients, complete ablation of metastatic deposits may be curative [8,9].

Electrolysis is a form of local ablation that uses direct current to produce tissue destruction. It does not rely on thermal effects at low currents [3,11–15] and has been shown to be safe, predictable and effective in a linear dose-related manner [16–18]. The use of small metallic electrodes makes electrolysis suitable for percutaneous use.
We have successfully ablated liver metastases in patients and have reported complete ablation of such tumours using electrolysis, which was confirmed histologically after 1 year [18]. By placing an electrode catheter into the centre of a colorectal metastasis, electrolysis will result in local cell necrosis around the anode and cathode, and potentially provide a simple and safe ablative technique for patients who may have unresectable disease.

Electrolysis is currently limited by the lack of an effective real-time monitor of the extent of induced necrosis, with a pre-determined ablative dose being calculated on the basis of historical data [3,19]. Development of a real-time monitor would allow confident and accurate tumour destruction during electrolysis without excessive loss of normal tissue.

Electron transfer during electrolysis polarizes the tissue in order to complete the circuit between the implanted
Numerous chemical reactions occur as a result, and differential hydrogen ion concentrations form at the electrodes according to the following equations:

Anode: \(2\text{H}_2\text{O} → \text{O}_2 + 4\text{H}^+ + 4e^-\)

Cathode: \(2\text{H}_2\text{O} + 2e^- → \text{H}_2 + 2\text{OH}^-\)

Consequently, the anode becomes acidic and the cathode alkaline. This differential pH was investigated as a possible real-time monitor of the extent of hepatic necrosis induced by electrolysis. The aim was to compare absolute pH values created by electrolysis with the histological appearance of normal pig liver.

**METHODS**

Local Animal Ethics Committees (University of Adelaide, SARDI/PISA and The Queen Elizabeth Hospital, Adelaide) approved the use of laboratory animals in this study. The study conformed to the Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC/CSIRO/AAC 1990) and the SA Prevention of Cruelty to Animals Act 1985.

Direct current was applied to normal pig liver, and the pH of the parenchyma 8 mm from the electrodes was recorded until a pre-determined pH was reached. The position of the pH probe was marked and compared with the appearance of the liver on histology.

A total of 14 domestic white pigs (of approx. 30 kg body weight) were used. After ketamine/xylazine sedation (20 mg/kg and 1.5 mg/kg respectively), the pigs were anaesthetized with 1.5% halothane via a laryngeal mask airway [23]. All animals underwent a midline laparotomy, with intra-operative monitoring by a pulse oximeter attached to the tail.

A 6 French platinum electrode catheter, comprising four platinum electrodes evenly spaced from the tip, was used (Cordis Webster; Johnson and Johnson Medical Pty. Ltd, North Ryde, NSW 2113, Australia). The electrode under study (anode or cathode) was situated in the middle of the three proximal electrodes. The catheter was placed in the liver so that this electrode was just under the liver capsule. The electrode at the tip of the catheter not under study was therefore 11 mm deep from the liver surface. The electrodes were connected to a purpose-built direct-current generator (Bioengineering, Transducers and Signal Processing Research Group, University of Leicester, U.K.) that was designed to deliver a constant current (80 mA) to a pre-determined ‘dose’ (in coulombs) by automatically varying the voltage (1 C = 1 A × 1 s).

At a distance of 8 mm and directly facing the electrode, an antimony monocrystal pH probe with external reference (Zinetics 24ME Multi-use pH catheter; Medtronic, Salt Lake City, UT, U.S.A.) was inserted with the sensor at a depth of 1 mm. The pH probe was calibrated against proprietary solutions of pH 1 and pH 7 (Medtronic) for acidic readings, and against a phosphate solution of pH 9.2 for alkaline readings. The probe was kept moist in the peritoneal cavity between readings. A pH recorder (Digitrapper MKIII; Synetics Medical, Stockholm, Sweden) was used to read the pH prior to electrolysis and then after every increment of 10 C until the pH began to change or the electrolytic lesion was visible on the surface of the liver and close to the pH recording site, at which point readings were recorded at 5 C increments. The direct current was disconnected for each pH reading to avoid interference of the current with the electropotential of the pH probe, and equally to prevent the metallic pH probe entering the electrical field and possibly acting as an electrode (Figure 1).

Several (between three and six) electrolytic lesions were made in each liver. When the pH had reached a pre-determined reading, the current was stopped entirely and the pH probe was removed. The pH was then recorded at 5 C increments. The direct current was then reconnected for each pH reading with the lesion visible on the surface of the liver. The pH probe was then removed and the lesion was marked with dye.
Liver surface Electrode hole pH hole (Alcian blue marker)

Figure 2  Histological grading of the extent of electrolytic lesion

Grade A, necrosis past pH/Alcian Blue hole; grade B, necrosis up to but not beyond the pH hole; grade C, necrosis short of the pH hole; grade D, no necrosis; grade O, no Alcian Blue seen. The liver was sectioned perpendicular to the liver through the plane of both pH and electrode holes.

the hole left by the pH probe was marked with Alcian Blue dye (Table 1). Lesions were created to reach pH values at increments of 0.5 pH units between 6.5 and 3.0

at the anode and between 8.5 and 10.4 at the cathode. As the experiment progressed, the pH under study was narrowed to values close to the normal range.

Electrodes were removed and the pigs were given analgesia (buprenorphine 0.1 mg/kg) before closing the abdomen in two layers (one polydioxanone and 3/0 monocryl) and waking the pig. The livers were harvested 3–4 days later, when preliminary studies have shown that electrolytic lesions attain maximum dimensions. The lesions were then separated and fixed in 10% buffered formalin for up to 1 week, before being sectioned in the plane of the electrode and pH holes, perpendicular to the surface of the liver. After embedding in paraffin, the samples were stained with routine haematoxylin and eosin. All specimens were analysed histologically by a pathologist, who was blinded to the experimental method, and graded A–D, defined as follows: A, necrosis past the pH probe; B, necrosis up to but not beyond the pH hole; C, necrosis short of the pH hole; D, no necrosis seen (Figure 2 and Figure 3a–3c).

Figure 3  Histological grading of created lesions

(a) Example of grade A histology. Alcian Blue is seen in the pH hole, surrounded by necrotic tissue. The necrosis has extended past the pH probe hole, and this is therefore a grade A lesion. (b) Example of grade B histology. This lesion was graded B, as the necrotic tissue is up to but not beyond the Alcian Blue. (c) Example of grade C histology. The Alcian Blue is in a thin area of necrosis, but has viable liver lobules either side of it. There is therefore a discontinuous lesion between the electrolytic necrosis and the pH probe, and this equates with a grade C lesion. (d) Histology of the edge of an anodic lesion. A rim of neutrophils is visible, then an area of congestion surrounds the eosinophilic necrotic zone on the right. To the extreme left of the picture is normal hepatic parenchyma. Original magnification: (a) × 4, (b)–(d) × 10.

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Four control animals were subjected the same procedure except that no current was delivered. Electrodes were left in situ for 20 min, with pH read every 2 min (corresponding to the delivery of 10 C).

Significant methodological problems were encountered, and 17 lesions were excluded prior to analysis. Four lesions were not harvested due to technical problems with the method (incorrect pH calibration in two lesions, pH meter failure and failure to stop the experiment at the pre-determined pH). In eight lesions no Alcian Blue was seen with histology, in three the pH probe lost contact and required re-calibration, and in one the pH probe hole bled after repeat trauma and the probe was moved. One specimen was lost.

In a further five lesions electrolysis was stopped early because of protracted anaesthesia (in two lesions) or erosion of the electrode catheter and loss of the circuit (three lesions). These lesions were analysed and are discussed.

RESULTS

In total, 14 pigs were used and 70 lesions were created (40 at the anode, 25 at the cathode and five controls). One pig died of hyperthermia, as an idiosyncratic reaction to anaesthesia, before a lesion was made. All other animals survived and had uncomplicated recoveries. Following exclusion of 17 lesions from the analysis due to methodological problems (see above), there were 30 anode, 19 cathode and four control lesions available for analysis.

The range of pH readings obtained prior to electrolysis was 6.5–8.7 (mean 7.36; median 7.4), and this was taken as the ‘normal’ range.

Histology

A central cavity, corresponding to the position of the electrode, was surrounded by variable amounts of ‘cellular debris’. This, in turn, was surrounded by necrosis, characterized by featureless, eosinophilic hepatocytes lacking glycogen vacuoles. There was invariably a sharp demarcation between this necrotic tissue and an actively proliferating surrounding zone, with fibroblast and biliary proliferation and a mixed picture of white-cell infiltration. Predominantly neutrophils were found, but often macrophages and giant cells were already present. Beyond this, a ‘congested’ zone occurred in some specimens (Figure 3d). On several sections there was a subcapsular rim of necrosis tapering away from the main lesion. This rim was only a few cells thick in all except one section (see ‘Anode’ section and Discussion), where it contained the Alcian Blue dye and contributed to the only false-positive result. In addition, the control lesions showed a very small rim of necrosis around the electrode and pH probe, presumably from the local trauma.

Anode

All lesions reaching a pH of 6 or less were necrotic at the pH probe or beyond (grade A or B) on histology (Figure 4). Two lesions were stopped without the desired pH reading, as the pigs showed some instability from a long anaesthetic. Both of these lesions revealed necrosis up to the pH probe hole and were consequently falsely negative. A further lesion that registered a pH change to 5.5 was falsely positive, as the pH probe was in a rim of necrotic subcapsular liver with no deeper necrosis corresponding to the electrode lesion. Between pH 6 and 6.5, two of nine lesions failed to show necrosis to the probe (grade C) and were not explained by the Alcian Blue being in a subcapsular necrotic rim.

Cathode

All lesions reaching a pH of 9 or greater were necrotic at or beyond the pH probe (grade A or B) (Figure 5). Of the five lesions registering between pH 8.5 and 9, three were necrotic at or beyond the probe (grade A or B) and two showed necrosis, but this was short of the pH probe (grade C) (Figure 3c). Three lesions were discontinued without reaching their desired pH because of erosion of the electrode. They had not reached their target pH of 9.
or more, but all passed pH 8.5 at some stage. None of these lesions were necrotic to the pH probe (grade C).

**Controls**

Five control pigs had electrodes inserted but no current passed. There was no evidence of necrosis in one (grade D). Three other animals showed a small rim of necrosis consistent with trauma from insertion of the instruments, but no necrosis resembling that of the specimens that had undergone electrolysis. The fifth specimen was lost before histological section.

**DISCUSSION**

Electrolysis is emerging as a safe, predictable and effective method of ablating colorectal liver metastases [3,17,18,24]. While there is a linear relationship between the number of coulombs delivered and the volume of necrosis induced, this relationship is prone to some inherent inaccuracies. That there must be a range of volume of necrosis per coulomb is predictable given the number of possible electrical conductors and resistors that are within the liver. For instance, the walls of blood vessels are good insulators of plasma, which in turn is a good conductor of electricity which can alter the extent of necrosis [25]. For these reasons, it is necessary to develop a real-time monitor of the effects of hepatic electrolysis, so that tumours can be ablated with greater predictability and without excessive loss of normal parenchyma. In the presence of neoplastic tissue, with disorganized tubular structures and sclerotic connective tissue, rates of necrosis are likely to be more difficult to predict. Given that the removal of a margin of normal tissue around a tumour is thought desirable [4,26,27], the use of pH readings in normal parenchyma, as in the present study, may have clinical application.

The cellular cause of necrosis is unknown. It is probably due to a combination of cytotoxic chemical production, pH changes and membrane disruption, although ischaemia and other host factors may also play a role [13–22,24,28–31]. Protons diffuse for a greater distance than the other chemical products of electrolysis and may well be responsible for the peripheral limit of destruction [19,21]. Measurement of pH is therefore rational as a means of real-time monitoring of electrolysis to determine the leading edge of the products of electrolysis. As cell death may not be solely pH-dependent, it is necessary to determine the pH that corresponds to cell death. The two false-negative cases in this series indicate that cell death may occur without deviation from the ‘normal’ pH change.

The assessment of anodic lesions was more reliable than that of cathodic lesions, as the pH probe is specifically designed to measure acidic pH. Anodic lesions characteristically desiccate, becoming well demarcated [21,28]. Conversely, cathodic lesions attract water and swell [21,28]. This may dilute and buffer the alkaline products and distort the margin of necrosis. The alkaline calibration solution used in the present study had pH of 9.2, and pH values more alkaline than this may have been inaccurate. However, as all specimens with a pH greater than 9 were necrotic, more refined accuracy is probably superfluous. The possibility of altering electrode configurations for electrolysis (e.g. central cathode surrounded by anodes) is under investigation [32] as a method of delivering electrolysis, and may allow standardization of the pH cut-off.

The ‘normal’ range of pH readings during the present study was surprisingly wide, at 6.5–8.7. The range of naturally occurring interstitial pH values is 6.4–7.55 [33,34]. The electrochemical reactions underlying the measurement of pH with antimony electrodes are largely unknown, but antimony corrosion is thought to generate a readable potential [35,36]. The phosphate buffer used for alkaline calibration also corrodes the crystal [35]. In addition, antimony monocrystals are known to be oxygen sensitive [35–37]. Aging (i.e. corrosion of the probe) and local fluctuations in oxygen tension may therefore contribute to the wide range of ‘normal’ values observed. Repeated insertion of pH probes has also been noted to add to inaccuracy [38]. On the single occasion that the pH probe hole began to bleed after repeated insertions, the pH was noted to increase, and it is assumed that variable capillary blood flow also affects interstitial pH until such time as the area becomes ischaemic from the advancing lesion. Given the wide ‘normal’ range, it is not surprising that pH values between 6.0 and 6.5 at the anode and between 8.5 and 9.0 at the cathode were inconsistently associated with cell necrosis.

The two false-negative outcomes (normal pH values associated with necrotic tissue) may have been the result of persistent circulation at the periphery of the lesion. Our observations suggest that large-calibre vessels (cut-off unknown) appear to be protected from the effects of electrolysis by the flow of blood, whereas smaller vessels will thrombose and contribute to the ablative process [15,24,28,30,31]. In the present experiment, it was noted that as the edge of the lesion approached the pH probe, the pH would fluctuate before returning to the normal range. This occurred for 5–10°C until a persistent change indicated necrosis. This observation is consistent with homoeostatic buffering at the periphery of the lesion by blood flow prior to necrosis.

The false-positive result (acidic pH value associated with viable tissue) was caused by the pH probe recording in a thin rim of subcapsular necrosis, discontinuous with the main electrode lesion (Figure 3d). Frequently, the cytotoxic effervescent fluid from the anode (chlorine, hydrochloric acid and hypochlorous acid) is seen tracking...
along the liver capsule with gravity, and there is invariably a small amount of capsular necrosis taping away from the lesion proper.

A pH probe has methodological limitations associated with its use, and it is not an ideal clinical tool. Nonetheless, the present study has demonstrated that tissue reaching pH values of below 6 at the anode and above 9 at the cathode reflects cell death with a high degree of specificity (95% for the anode and 100% at the cathode). The use of pH as a real-time monitor of electrolysis is thus rational and feasible.

Magnetic resonance spectroscopy measures cellular pH with a high degree of accuracy by extracorporeal continuous monitoring [39–42], and would provide a less crude method of pH monitoring during electrolysis. This technique would avoid major inaccuracies associated with our method, as it is atraumatic and is not dependent on local oxygen tensions, local blood flow or crystal corrosion. In addition, magnetic resonance spectroscopy would be compatible with the percutaneous use of electrolysis.

Normal human tissue has a physiological range of extracellular pH of 6.4–7.55 [33,34], whereas tumours tend to be more acidic [42,43]. If a rim of normal tissue is ablated as part of the process of electrolysis, the pH of the normal tissue will be the parameter determining the extent of necrosis. Physiological pH levels are unlikely to differ significantly between pigs and humans, but corroboration will be required before recommendations pertinent to electrolysis in humans can be made. The findings from the present study cannot be extrapolated to procedures using the Pringle manoeuvre, as conditions of global hepatic acidosis appear to protect against cell death to a degree [44,45].

In conclusion, the present study establishes that changes in pH can reliably predict cell death from electrolysis in normal pig liver. The pH values may vary with the equipment and method used, and further study with highly sensitive pH-measuring equipment (e.g. magnetic resonance spectroscopy) is necessary before absolute values equating with cell death can be recommended. Corroboration with pH changes during electrolysis in normal and tumorous human tissue is also necessary in order to develop pH as a monitor in the clinical situation. These studies are under way.

REFERENCES


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