A new treatment for unresectable liver tumours: long-term studies of electrolytic lesions in the pig liver

Simon A. WEMYSS-HOLDEN, Gavin S. M. ROBERTSON, Ashley R. DENNISON, Paula S. VANDERZON, Pauline de la M. HALL and Guy J. MADDERN
University of Adelaide, Department of Surgery, The Queen Elizabeth Hospital, Woodville Road, Woodville, South Australia, 5011, Australia

ABSTRACT

The majority of liver tumours are inoperable and an alternative treatment to surgical resection is urgently needed. Electrolysis has been investigated in a rat model and the procedure is safe, with accurate and predictable effects. The necrosis produced has also been shown to cause destruction of tumour deposits in the rat liver. A similar evaluation in a large animal model was necessary before clinical trials could commence. Using platinum electrodes connected to a d.c. generator, areas of hepatic necrosis were created in the pig liver. Animals were killed at various time points after treatment to assess the extent of healing. Treatment was uneventful and all animals made a full recovery. No animal died from the treatment or had to be killed prematurely. After 2 days of treatment, healing was minimal but at successive time points there was progressive evidence of healing, such that after 4 months, the original electrolytic lesion was greatly reduced in size and the large area of necrosis seen at the early time points was largely replaced by a fibrous scar with only small islands of necrotic tissue. In a large animal model, electrolysis is a safe method for creating areas of hepatic necrosis. The lesions heal with time and are associated with minimal morbidity. The results support a trial of electrolysis in patients with unresectable liver tumours.

INTRODUCTION

The majority of malignant liver tumours remain inoperable due to their number, distribution or the presence of parenchymal disease. Following apparently curative surgery for colorectal cancer, half the patients will develop hepatic metastases [1]. Under these circumstances liver resection, when appropriate, significantly improves survival with between 25 and 35% of patients being alive at 5 years [2,3]. Unfortunately only 5–10% of patients who present with liver metastases are suitable candidates [4] and in the remainder the outlook is dismal [5–7]. The situation is similar for patients with hepatocellular carcinoma with a resectability rate of less than 20% [8], although when surgery is feasible the 5 year survival rate approaches 50% [9]. Again, however, the survival in untreated patients is extremely poor with a median survival of approximately 4 months and a 3 year survival of less than 15% [10,11].

Clearly a treatment is needed that is applicable to patients who are unsuitable for a surgical approach but which, like surgery, would ideally not only provide symptomatic relief but would be able to improve the outlook due to reduction or eradication of the tumour(s). A number of modalities have been investigated including cryotherapy [12,13], alcohol injection [14], interstitial laser therapy [15] and others. To date none has proved to be safe and minimally invasive, while at the same time #
producing a controllable, predictable area of destruction, within which survival of tumour cells is impossible. As a consequence, no presently available treatment has been shown to confer any advantage in terms of disease free interval or survival.

Previous experimental work from this Unit [16,17] demonstrated that electrolysis has considerable potential for treating unresectable liver tumours. In the rat, electrolysis is a safe, effective and controllable treatment. The ellipsoidal zones of coagulative necrosis heal with time and are associated with no long-term morbidity.

Although electrolysis uses electrical current to cause tissue necrosis, its mode of action is new and completely unlike diathermy, laser or cryotherapy where changes are principally due to the thermal effect [17–19]. A small direct electrical current (< 50 mA) is passed between two electrodes which become polarized. A pH gradient is established [20,21] and cytotoxic electrode products are liberated [22–26]. Well defined zones of tissue necrosis are produced around the tip of each electrode.

Nevertheless, progression to the clinical setting requires further evaluation of the method of production of the electrolytic lesion and its evolution in an appropriate large animal model. The aim of this study was to extend the work by a detailed study of the immediate effects and evolution of large electrolytic lesions in the pig, including long term studies.

This study examined the safety, morbidity and rate of healing of electrolytic lesions in the pig liver.

**METHODS**

The d.c. generator used in this study was manufactured by the Bioengineering, Transducers and Signal Processing Research Group (University of Leicester, U.K.). Once connected to platinum electrodes inserted into the liver parenchyma, it was used to deliver an electrolytic ‘dose’ of 100 C (coulombs) at 50 mA.

Electrode catheters were supplied by Johnson & Johnson Medical Pty Ltd (North Ryde, NSW, Australia). The electrode catheters were 6 French gauge (2 mm diameter). Each catheter had three platinum electrodes proximal to the platinum tip electrode (anode; Figure 1). Any one of the three proximal electrodes could be selected and used as the cathode (negative electrode).

The use of laboratory animals in this study was approved by the local Animal Ethics Committees (University of Adelaide, South Australian Research and Development Institute, Pig and Poultry Production Institute of South Australia and The Queen Elizabeth Hospital, Adelaide), and the study conformed with the Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, Commonwealth Scientific and Industrial Research Organisation, Australian Agricultural Council, 1990) and the South Australian Prevention of Cruelty to Animals Act 1985.

Sixteen female specific-pathogen-free domestic white pigs (28.5 to 32.0 kg) were obtained from the Pig and Poultry Production Institute (Roseworthy Campus, Roseworthy, SA, Australia). The animals were housed in group pens (max. 10 animals per pen) and had access to a single space wet/dry feeder and ad-libitum water. The pens were maintained at 23.0 ± 1.0 °C at ambient humidity. Preoperatively, the animals were fasted for 12 h.

**Experimental protocol**

In order to establish the rate of healing and morbidity associated with electrolysis, the 16 animals were divided into four groups:

- **Group 1**: four animals were treated with 100 C and killed 2 days after treatment,
- **Group 2**: four animals were treated with 100 C and killed 2 weeks after treatment,
- **Group 3**: four animals were treated with 100 C and killed 2 months after treatment,
- **Group 4**: four animals were treated with 100 C and killed 4 months after treatment.

**Operative technique**

Animals were sedated with a deep intra-muscular injection of ketamine (20 mg/kg) and xylazine (1.5 mg/kg) and spontaneous breathing general anaesthesia was maintained with 1.5% halothane in 100% oxygen. Each animal was given a perioperative intramuscular injection of antibiotics (teramycin).

To determine the effects of electrode separation on healing and morbidity each animal was anaesthetized once and hepatic lesions were created with electrodes together (12 mm separation, single lesion produced) and apart (200 mm separation, two lesions produced). Each
animal therefore had three discrete electrolytic lesions in total.

The liver was exposed using an upper midline incision. A single electrode catheter was inserted into the dome of the right lobe to a depth of 30 mm. The electrodes were connected to the d.c. generator and a ‘dose’ of 100 C was delivered to the tissue. The resulting lesion was called the ‘composite’ lesion, as it consisted of two distinct but overlapping (anode and cathode) lesions.

Two separate electrode catheters were then inserted with the anode peripherally in the right lobe and the cathode in the centre of the left lobe, producing an electrode separation of 200 mm. The tip electrodes of each catheter were connected to the d.c. generator and 100 C was again delivered to the tissue. After treatment the electrodes were disconnected and the catheters were removed. The wound was irrigated with aqueous Betadine, and the abdomen was closed in two layers with 1 polydioxanone and 3/0 monocryl. Each animal was then returned to a single pen.

Blood samples were obtained preoperatively, at 1 day and 1 week postoperatively, and at the time the animal was killed. Serum measurements of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, γ-glutamyltranspeptidase (γGT) and bilirubin were made on each sample (Technicon AXON analyser, Bayer Health Care, Pymble, NSW, Australia). Pre-operative values were compared with those at 1 day and 1 week postoperatively, and at the time the animal was killed.

Once the wounds were healed, the animals were returned to group pens. All animals were killed by lethal injection (lethabarb).

Liver enzymes
Liver enzymes (AST, ALT and γGT) were significantly elevated on the first day after treatment (P < 0.001, 0.001 and 0.01 respectively). In group 1 (killed 2 days after treatment) the AST was still significantly elevated at the time the animal was killed (P < 0.05). In long term studies (groups 2, 3 and 4) liver enzymes had returned to normal one week after treatment and remained so until the animal was killed.

Liver pathology
Group 1 (2 days after electrolysis)
At autopsy fine, fibrinous adhesions were present between the liver and diaphragm in two animals. The abdominal cavity and organs other than the liver were otherwise normal in all four animals. The livers were macroscopically normal apart from small puncture marks at the sites of electrode insertion. There were no liver infarcts. Macroscopic examination of the electrolytic lesions showed discrete, spherical zones of hepatic necrosis, sharply demarcated from the adjacent normal liver (Figure 2). Histological examination of the electrolytic lesions confirmed extensive but well-defined spherical areas of coagulative necrosis surrounding the site of the electrode tips. Small numbers of proliferating fibroblasts, a mild mononuclear cell infiltrate, and bile ductular proliferation were seen at the junction between the electrolytic lesion and the surrounding liver tissue. The immediately adjacent liver showed focal areas of haemorrhage but no other pathology (Figure 3, upper left panel).

Group 2 (2 weeks after electrolysis)
Mean weight gain was 7.4 kg (range 6.0 to 9.5 kg). Two animals developed mild superficial wound infections that responded rapidly to antibiotics (penicillin). One animal
Figure 3  Section of pig liver showing the appearance 2 days (upper left panel), 2 weeks (upper right panel), 2 months (lower left panel) and 4 months (lower right panel) after treatment

(Upper left panel) An extensive but well-defined area of coagulative necrosis (CN) is seen around the site of the electrode tip. A few proliferating fibroblasts (F), a mild mononuclear cell infiltrate and proliferating bile ductules are seen at the junction between the electrolytic lesion and the surrounding liver tissue (NL). The immediately adjacent liver shows areas of focal haemorrhage but no other pathology. (Upper right panel) A large confluent central area of coagulative necrosis (CN) is seen with a peripheral rim of proliferating fibrous tissue (F) and ingrowth of fibroblasts. There is a small amount of focal dystrophic calcification in the lesion. (Lower left panel) There is evidence of advanced healing, with areas of proliferating fibrous tissue (F), intermingled with small islands of residual necrotic tissue (CN); adjacent normal liver (NL). (Lower right panel) There are only small islands of residual necrotic tissue (CN) intermingled with large amounts of mature fibrous tissue (F).

suffered a partial superficial wound dehiscence with abscess formation which was successfully treated with wound toilet and antibiotics. The deep closure remained intact. All animals were healthy at the time of their death. At autopsy, fibrinous adhesions were present in all of the animals between the liver and diaphragm but were easily divided. The livers were otherwise macroscopically normal with no evidence of infarction. The sites of electrode insertion could not be identified. Histological examination showed a large confluent central area of necrosis with a peripheral rim of proliferating fibrous tissue and some proliferating bile ductules, the adjacent liver was otherwise normal. Small amounts of focal dystrophic calcification were occasionally seen in the lesions (Figure 3, upper right panel).

Group 3 (2 months after electrolysis)
Mean weight gain was 43.0 kg (range 36.0 to 48.0 kg). One animal developed a deep wound infection with abscess formation 3 weeks after treatment. The infection responded to wound toilet and antibiotics and the skin healed. However, 5 weeks after treatment it was evident that the deep closure had partially dehisced as the animal developed a wide-necked incisional hernia. The hernia was treated conservatively and was uncomplicated. The animals were healthy at the time of their death. At autopsy, dense adhesions were present between the liver and diaphragm in three of the animals. The abdominal cavity was otherwise normal. The livers were of normal size and macroscopically normal. Histological examination of the electrolytic lesions showed variable degrees of advanced healing, usually with areas of proliferating fibrous tissue, intermingled with small islands of residual necrotic tissue and focal bile ductular proliferation at the junction between the area of necrosis and the adjacent normal liver (Figure 3, lower left panel).

Group 4 (4 months after electrolysis)
Mean weight gain was 80.3 kg (range 73.0 to 88.5 kg). Six weeks after treatment, one animal developed an incisional
hernia secondary to a deep wound infection. The hernia was repaired under general anaesthesia to prevent complications. The animal made an uneventful recovery. All of the animals were healthy at the time of their death. In all four animals dense fibrous adhesions were again evident at autopsy but laparotomy was otherwise normal. Histological examination of the electrolytic lesions showed even more advanced healing, with large amounts of mature fibrous tissue intermingled with small numbers of foreign body giant cells and haemosiderin-laden macrophages (Figure 3, lower right panel).

**DISCUSSION**

Having previously shown that electrolysis is a safe, predictable and reproducible method for creating discrete areas of hepatic necrosis in a rat model [16], this study was instigated to establish the safety of electrolysis in a large animal model prior to clinical trials. ‘Safety’ was determined by observing the extent of healing of the induced electrolytic lesions at several time points after treatment, and any treatment related morbidity or mortality.

All of the animals tolerated the treatment well. There were no intraoperative complications associated with the electrolysis and post-operative recovery was universally rapid and uneventful. In this study electrolysis appeared to be associated with minimal initial systemic trauma. In the clinical setting, inadvertent insertion of the catheter into a hepatic vein would be extremely unlikely, due to the use of intraoperative imaging (ultrasound or magnetic resonance imaging) to place the electrode within the tumour. The action of the cytotoxic electrode products would therefore remain localized. However, further studies are currently being performed to establish the systemic effects of the ‘worse-case scenario’ where the electrodes are placed within the lumen of a hepatic vein and all of the electrode products pass directly into the inferior vena cava. If this extreme situation is well tolerated, it is reasonable to assume that treatment of patients under more controlled conditions would be safe and associated with a minimal level of complications.

Liver enzymes were elevated immediately after treatment but this increase was small and transient. This reflects the initial release of enzymes from ’leaky’ injured cells prior to the death of these cells. It also supports our belief that there is no ongoing hepatic ischaemia or necrosis after the initial electrolytic insult in the pig. This finding differs from a previous similar study in the rat [16] where most of the ‘primary’ electrolytic lesions were associated with a peripheral infarct, an effect which was caused by vascular occlusion resulting from a relatively large ‘primary’ lesion in a physically small lobe. As the pig liver is much larger, the parenchyma peripheral to the ‘primary’ lesion is more likely to survive the electrolytic insult because of its collateral supply. The adult human liver is again larger than that of a 30 kg pig and extrapolation of these results would suggest that peripheral infarction in the clinical setting is unlikely although clearly not impossible for large peripherally placed lesions.

Postoperatively, all animals gained weight at a normal rate and remained clinically healthy until they were killed. Five animals developed post-operative wound infections despite antibiotic prophylaxis. All infections responded well to conservative treatment but in one of the two animals that developed incisional hernias it was decided to repair this surgically as it was an animal assigned to the 4 month survival group. The surgery was uneventful but in retrospect it may have been advantageous to keep the animals in individual pens as hygiene in a group pen is difficult although stress levels are reduced when the animals are group housed. Apart from wound problems, no infectious complications were encountered. No animal became clinically septic, developed overt cholangitis or peritonitis.

When patients are treated, secondary infection is a concern in any situation where tissue necrosis is produced but infection in the liver was not a feature of any of the 48 areas of electrolytic necrosis produced in this study. This was probably because the local environment around the electrodes is sterilized during treatment by the cytotoxic electrode products. Platinum salts, leached from the electrodes during treatment may also augment this effect as they are strongly bactericidal even in low concentrations [27,28]. This contributes to the extremely low long-term morbidity associated with electrolysis and suggests that there are unlikely to be any major mid- or long-term complications in the clinical setting.

In each treatment group the composite lesion was larger than either the anode or cathode lesions, but the overall pattern of healing was the same in each of the lesions with progressive replacement of the central area of coagulative necrosis by fibrous tissue. All lesions progressively decreased in size although at 4 months more necrotic tissue remained in the centre of the composite lesions than in the smaller anode and cathode lesions. Healing occurred by proliferation and ingrowth of fibroblasts from the periphery of the lesions. Apart from focal areas of haemorrhage in some of the livers at the early time points, the liver parenchyma immediately adjacent to the lesions was entirely normal. The hepatic trauma associated with electrolysis appears to be short-lived and confined to the initial insult which results at the time of treatment (there was no evidence of ongoing or progressive hepatic necrosis). These findings in normal liver tissue suggest that tumours treated by electrolysis should heal in a similar way. An appropriate electrolytic ‘dose’ would be selected to not only completely encompass the tumour, but also to cause necrosis of a small rim of surrounding normal liver tissue. It is suggested...
that healing of an area of tumour necrosis should differ little from the healing of necrotic normal liver as healing would take place from the periphery of the lesion by the ingrowth of fibroblasts from the surrounding normal parenchyma.

Platinum electrodes were used in this study as they are not obviously corroded by the electrolytic process [25] and may confer a theoretical, albeit unproven, benefit from the liberation of small concentrations of cytotoxic platinum salts [25,27–31]. If any new treatment for unresectable liver tumours is to be used clinically and be widely applicable, it must not only be safe and effective but also relatively inexpensive. In the clinical setting, electrode delivery systems would need to be disposable. It is envisaged that several electrodes would be inserted into single or multiple tumours under local anaesthetic and imaging control (ultrasound, computer-generated tomography or magnetic resonance imaging) and patients could be treated on an out-patient basis (with follow-up imaging). If the electrodes were made of platinum, the expense of such disposable systems may be prohibitive. Preliminary studies suggest that other electrode materials, such as graphite may prove to be equally effective and considerably less expensive.

This study in a large animal model has shown that discrete areas of hepatic necrosis induced by electrolysis are created in a safe way with minimal immediate, mid- or long-term morbidity. The induced lesions heal with time. The results support clinical trials in patients with unresectable liver tumours who are at present incurable.

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REFERENCES


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