

## ORIGINAL ARTICLE

# ELECTROCHEMICALLY INDUCED HEPATIC NECROSIS: THE NEXT STEP FORWARD IN PATIENTS WITH UNRESECTABLE LIVER TUMOURS?

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**Background:** The treatment of patients with unresectable liver tumours remains an unsolved clinical problem. Several methods of loco-regional treatment have been developed. These methods rely mainly on direct thermal or chemical insults and consequently have their own inherent limitations in clinical usage. The 'ideal' treatment would combine the direct cytotoxic effects of chemical treatments with the relative predictability of thermal insults, without the associated complications. This study aims to investigate whether the direct chemical effect of electrolytic hepatic necrosis is associated with any heating effect, and if so, whether the temperature change is dose-dependent. **Methods:** An electrolytic 'dose' sufficient to induce a localized zone of hepatic necrosis was delivered to the livers of rats and pigs via implanted platinum electrodes.

**Results:** The results showed that there was no significant temperature increase at low current levels (2-4 mA) in the rat liver. In the pig, there was a significant ( $P < 0.01$ ) increase in temperature of 4.2°C during electrolysis, when delivered at between 20 and 50 mA. However, such a small increase in temperature would have been insufficient to cause appreciable thermal necrosis.

**Conclusions:** This study demonstrates that electrolysis-induced hepatic necrosis is produced without an increase in temperature; clearly cell death results from the direct effects of cytotoxic electrode products and an alteration of intracellular pH. Consequently, it is likely that as a method for ablating liver tumours, electrolysis should be associated with fewer complications than other forms of locoregional treatment.

**Key words:** electrolysis, liver, thermal, tumour.

## INTRODUCTION

The majority of patients who develop hepatic metastases of colorectal origin are incurable. Only 5-10% of these patients are suitable candidates for surgical resection,<sup>1</sup> which is the only treatment that has been shown to improve survival.<sup>2</sup> For the remainder, median survival is 6 months and an effective treatment for these patients is urgently needed.<sup>3</sup>

Several methods of local treatment for unresectable liver tumours have been developed. Many of these techniques, such as interstitial laser therapy,<sup>4</sup> cryotherapy,<sup>5</sup> and microwave hyperthermia,<sup>6</sup> cause tissue necrosis by delivering a direct thermal insult to the tumour. Other methods use the direct cytotoxic effects of certain chemicals such as alcohol,<sup>7</sup> and chemotherapeutic drugs.<sup>8</sup> However, the effects of these are largely unpredictable and uncontrollable. Thermal methods of tumour ablation have certain inherent limitations, and dangers such as fracturing the ice ball with potentially fatal haemorrhage (cryotherapy), carbonization and charring (interstitial laser therapy) would not be encountered if tissue destruction could be produced at ambient temperature. Theoretically, the 'ideal' treatment would combine the relative predictability of the thermal treatments with the direct cytotoxic effects of the chemical methods, without their individual drawbacks.

Electrolysis is a new local treatment for patients with unresectable primary or secondary liver tumours which is currently

being developed for use in patients at the Queen Elizabeth Hospital. Early results suggest that this technique incorporates the advantageous properties of chemical and thermal treatments without the associated complications. Localized tissue necrosis is produced by passing a low-voltage direct current (DC) between anode (positive) and cathode (negative) electrodes. When the current is applied *in vivo*, sodium hydroxide and hydrogen are produced at the cathode and hydrochloric acid, oxygen and chlorine gas at the anode.<sup>9-13</sup> This results in a significant pH gradient being established between the electrodes.<sup>14,15</sup> Consequently, the local environment becomes intensely cytotoxic and cell necrosis results. The resulting spherical zone of necrosis is sharply demarcated from the surrounding normal liver and the tissues between the electrodes are unaffected. Results in the rat and pig livers showed that electrolysis is a safe and effective method for creating areas of hepatic necrosis. Histopathology confirmed the presence of necrotic tissue at the electrode sites. Moreover, the induced necrosis was dose-dependent and was produced at a rate of between 2.0 cm<sup>3</sup>/100 coulombs (C) (rat) and 2.3 cm<sup>3</sup>/100 C (pig). It was proposed that the observed inter-species variation in rates of necrosis resulted from the difference in resistivity of the liver parenchyma between the two species (unpubl. observ.).

Because electrolysis uses electric current, it is reasonable to assume that cell necrosis may in part result from a heating effect. Were this so, it would potentially be associated with the complications of thermal treatments. A review of the literature on the temperature change associated with electrolysis showed variable results. Temperature changes ranging from 0 to 20°C have been reported.<sup>16-18</sup> However, all papers suggest that the thermal effect of electrolysis is negligible.

For electrolysis to be accepted as a new treatment method for

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patients with unresectable liver tumours it must be shown to be superior to those techniques currently available. As such it should be demonstrated that the direct electrochemical insult is delivered without a significant heating effect and the associated potentially life-threatening complications. Using both small and large animal models this study aimed to (i) determine if hepatic necrosis could be produced in the rat liver at low amperage with no associated temperature increase; and (ii) determine if thermally induced necrosis is likely to occur in the clinical setting where higher amperages will be employed using a pig model.

## METHODS

The direct current generator was manufactured by the Bioengineering, Transducers and Signal Processing Research Group, University of Leicester, United Kingdom. All experiments on animals were approved by the ethics committees of the University of Adelaide and the Queen Elizabeth Hospital and conform to the statements of Animal Experimentation by the NH&MRC.

### Rats

Sixteen SPF female Wistar WAG rats weighing a mean of 199 g (range 192–205 g) were anaesthetized using standard halothane/nitrous oxide/oxygen anaesthesia. Anaesthesia induction was performed using a perspex box with 3.5% halothane, 1 L/min nitrous and 1000 cc/min oxygen. Maintenance of anaesthesia was achieved with an inhalation system using 1.5% halothane, 0.5 L/min nitrous and 300 cc/min oxygen. The liver was exposed and everted through a midline incision. Two platinum electrodes (0.5-mm diameter) were inserted to a depth of 2 mm into one lobe of the rat's liver with a separation of 2 mm. A 0.8-mm-diameter thermocouple (80TK Thermocouple Module FLUKE used with Escort EDM-169S TÜV, Rheinland Group) was inserted into the liver between the electrodes with care taken not to touch the electrodes with the thermocouple. Core temperature was continuously monitored and recorded using a 3-mm-diameter rectal probe (KM250, Kane-May Limited).

Thermocouple and electrodes were placed into the liver and the equipment set up prior to randomization of treatment. The person recording the temperatures was not blinded to the treatment group.

Ambient environmental temperature was also continuously monitored and recorded using a temperature probe (HMP 35, Vaisala Pty Ltd, Helsinki, Finland) connected to an indicator unit (HMI, Vaisala Pty Ltd) placed next to the rat. Liver temperature at the electrode site, ambient environmental temperature immediately surrounding the rat and rectal temperature were all measured and recorded.

From previous work (G. Maddern *et al.* unpubl. data, 1998) it was shown that a current of 4 C produced a 5-mm-diameter lesion in the rat liver. The rate at which the dose could be delivered was altered by increasing or decreasing the current. In order to determine whether increased rate of delivery influenced the temperature, 4 C were delivered at both 2 and 4 mA. All rats were killed at the end of treatment by anaesthetic overdose.

Nine rats received electrolysis: in each rat one lobe of the liver was treated with 4 C at 4 mA, and the other lobe with 4 C at 2 mA. Seven rats were used as controls where every procedure and recording was identical to the treatment rats, but no current was passed between the electrodes. The mean time of each electrolysis treatment was matched with the temperature recording time for the

controls (18 min for 4 mA, 32 min for 2 mA) and both lobes of the liver were used.

### Pigs

Due to size limitations in the rat liver, pigs were used to deliver the electrolytic dose at higher currents similar to those that are used in the clinical setting to determine if there was significant heating around the electrodes.

Pigs were fasted overnight and sedated with a deep-intramuscular injection of ketamine (20 mg/kg) and xylazine (1.5 mg/kg). Pigs were maintained on gaseous anaesthesia of 1.5% halothane in oxygen via a laryngeal mask airway. Temperature studies were performed on the pigs at doses of between 20 and 50 mA (the range likely to be used in patients) by measuring core temperature, ambient temperature and liver temperature at the electrolysis site every 2 min for a total of 10 min at each setting. Temperature measurements were also continued for 2 min after the end of electrolysis to determine if any rises in temperature continued after the electrolysis had finished. All measuring devices and randomizing methods were the same as those used for the rat studies. The electrodes used were supplied by Johnson & Johnson Medical Pty Ltd, North Ryde, NSW. They were 6 French G with a diameter of 2 mm.

A total of five pigs were used for this analysis. Three pigs were controls with a total of eight 12-minute control studies performed. The remaining two pigs underwent thirteen 12-minute studies (six studies on one and 7 on the other).

A repeated measures analysis of variance was used to analyse the temperature change of the liver around the electrodes over time. Baseline measurements of rectal temperature, liver temperature and ambient temperature were included as covariates, and the rectal and ambient temperatures over time were included as time-varying covariates. An auto-regressive error structure was assumed.

Program 5 V from the BMDP statistical software package was used for this analysis by a consultant statistician.

## RESULTS

All animals tolerated the treatment well. No intra-operative complications were observed and no animal died prior to the anaesthetic overdose.

### Rats

The change in temperature around the electrodes was significantly different during electrolysis when compared with the control group ( $P < 0.01$ ). Interestingly, a mean temperature increase of 1.5°C was recorded in the control group; no temperature change was observed in the electrolysis group (Table 1). The site of electrolysis in the rat liver (left or right lobe) made no significant difference to the temperature changes.

### Pigs

There was also a significant difference in the temperature recordings in the pig liver between the treated and control groups. The baseline liver temperature increased by a mean of 4.2°C over the course of electrolysis and decreased by a mean of 0.7°C in the control group of animals where no current was passed between the electrodes ( $P < 0.01$ ) (Table 2). The mean temperature increase after electrolysis using 20 mA of current

**Table 1.** Results of the temperature changes in the rat liver (°C) at the site of electrolysis with the estimated mean values and the standard error of the estimate in parenthesis

	Estimated mean liver temperature at the start of electrolysis (°C)	Estimated mean liver temperature at the end of electrolysis (°C)	Temperature change	Significance
Electrolysis group	32.4 (0.22)	32.4 (0.35)	0	NS
Control group	32.4 (0.22)	33.9 (0.35)	1.5°C↑	P < 0.01

NS, not significant.

**Table 2.** Results of the temperature changes in the pig liver (°C) at the site of electrolysis with the estimated mean values and the standard error of the estimate in parenthesis

	Estimated mean liver temperature at the start of electrolysis (°C)	Estimated mean liver temperature at the end of electrolysis (°C)	Temperature change	Significance
Electrolysis group	41.0 (1.31)	45.2 (1.28)	4.2°C↑	P < 0.01
Control group	36.3 (1.66)	35.6 (1.63)	0.7°C↓	NS

NS, not significant.

was 2.6°C, 9°C with 30 mA, 8°C with 40 mA, and there was a 2°C mean temperature increase for the 50-mA group. Therefore the magnitude of the current did not significantly affect the temperature change over time. It is difficult to explain why there is a temperature difference at baseline between the control animals and the electrolysis animals. Operative conditions, theatre lights and length of anaesthesia all may contribute to this.

## DISCUSSION

This study has shown that with electrolytic doses which have been shown to kill tumours in the rat liver (G. Maddern *et al.* unpubl. data, 1997) the necrosis does not result from hyperthermia. Clearly, the mechanisms involved in tumour ablation are not thermal but are likely to result from the fiercely cytotoxic environment established around the electrodes during treatment. Specifically, sodium hydroxide and hydrogen are produced at the cathode and hydrochloric acid, oxygen and chlorine gas at the anode.<sup>9-13</sup> Chloride ions are attracted to the anode and are largely oxidized to chlorine gas; water is electrolysed releasing both ionic and molecular oxygen. Chlorine is a powerful oxidant which immediately attacks surrounding tissue.<sup>11</sup> As a result of these ionic fluxes, a pH gradient is established between the electrodes with the anode becoming acidic relative to the basic cathode, and a sharply demarcated zone of necrosis results.<sup>15</sup>

In the clinical setting, it is envisaged that relatively large liver tumours will be treated using electrolysis. This will require an electrolytic dose several orders of magnitude greater than that which was typically used in the rat (up to 1000 C). In order to maintain reasonable treatment times, this dose will need to be delivered at a higher current, as was used in the pig phase of this study. The maximum current is likely to be limited by patient compliance.<sup>19</sup> At up to 50 mA there was a statistically but not clinically significant increase in temperature of the local environment during electrolysis; an increase of 4.2°C would be insufficient to induce necrosis. Even at 100 mA temperature increases of only 7°C have been reported.<sup>18</sup>

Several methods of localized tumour necrosis rely upon a thermal insult to cause tissue destruction.<sup>4-6</sup> While excessive heat

or cold is undoubtedly effective and relatively predictable, both have been associated with certain complications such as haemorrhage, biliary fistulae and renal impairment,<sup>20</sup> carbonization and charring. Other methods that deliver a direct chemical insult to the tumour such as alcohol injection and locoregional chemotherapy are attractive but largely unpredictable in effect.<sup>7,8</sup> This study has shown that the directly delivered electrochemical insult of electrolysis is not associated with any significant heating effect and is therefore likely to be associated with a lower complication rate than thermal or chemical treatments when treating patients with unresectable liver tumours.

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